# Technical Review of Diisononyl Phthalate

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**Data Gathering and Analysis Division**
**and**
**Existing Chemicals Risk Assessment Division**

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## Purpose of the Technical Review of Diisononyl Phthalate

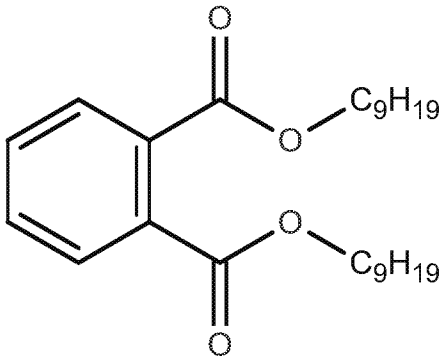
Before a chemical can be proposed for addition to or deletion from the EPCRA section 313 list, a hazard assessment must be performed for the chemical. The statutory chemical listing/delisting criteria of EPCRA section 313(d)(2) are generally based on hazard rather than risk. That is, the analysis is generally limited to the process of determining whether exposure to an agent can cause an increase in the incidence of a particular adverse effect in humans or wildlife. The information summarized in this technical review is intended to help petitioners and the public understand what data EPA considered in the assessment of DINP.

The technical review provides an overall assessment and review of a chemical's toxicity database which includes a review and consideration of both human health and environmental hazard. The technical review focuses on reviewing the scientific soundness of the readily available data or studies including an assessment of the severity of the effect(s) and the dose at which the effect(s) were observed. In the process of completing this technical review, the following technical reports are generated: chemistry profile; human health hazard assessment; environmental hazard assessment, including ecological toxicity and environmental fate; economics analysis; and, if relevant, an exposure assessment report.

With respect to human health effects, a chemical may be listed if it is known to cause or can be reasonably anticipated to cause 1) significant adverse acute human health effects at concentration levels that are reasonably likely to exist beyond facility site boundaries as a result of continuous, or frequently recurring releases or 2) cancer or teratogenic effects, serious or irreversible reproductive dysfunctions, neurological disorders, heritable genetic mutations, or other chronic health effects in humans. Current EPA risk assessment guidelines consider all manifestations of developmental toxicity, including fetal death, structural abnormalities, growth alterations, and functional deficits, to be of concern. Teratogenicity is a subcategory of the broader category "structural abnormalities."

## Contents

# Chapter 1
# Chemistry and Fate Assessment




## I. Executive Summary

Diisononyl phthalates (DINP) are the diisononyl esters of 1,2-benzenedicarboxylic acid (i.e., phthalic acid). DINP consists of any branched alkyl di-ester of 1,2-benzenedicarboxylic acid in which each alkyl ester moiety contains nine carbon atoms. The molecular formula for DINP is $C_{26}H_{42}O_4$. The structure of DINP is shown above with the nine-carbon atom, branched alkyl ester moieties represented by the molecular formula $C_9H_{19}$.

DINP constitutes a family of di-ester phthalates widely used as plasticizers (Kirk-Othmer, 1996). They are colorless, oily liquids with high boiling points, low volatilities, and are poorly soluble in water (less than one µg/L) (EPI Suite, 2017). Multiple CAS Registry Numbers are associated with DINP: 28553-12-0, 71549-78-5, 14103-61-8 and 68515-48-0 (Chemical Abstract Service (CAS), 2022; ChemIDplus, 2000; Elvers et al. 1992). There is no single generic CAS Registry Number that represents all DINPs. The chemicals represented by CAS Registry Numbers 28553-12-0 and 71549-78-5 consist of mixtures of isomers (compounds which have the same molecular formula but differ in the arrangement of their atoms). CAS Registry Number 14103-61-8 represents a single isomeric structure of DINP. Each of the alkyl ester moieties of the diisononyl phthalate esters represented by the three CAS Registry Numbers stated above are branched, and each contain a total of nine carbon atoms. In the above figure, the branched alkyl ester moieties are represented by the molecular formula $C_9H_{19}$. Although the molecular formulas of these nine-carbon alkyl ester moieties are the same for these DINP isomers, they differ in

structure due to the specific branching within the alkyl moieties. The chemicals identified by CAS Registry Number 68515-48-0 are also considered DINPs, but unlike the chemicals represented by the other three CAS Registry Numbers discussed above, 68515-48-0 consists of di-ester phthalates with nine-carbon alkyl ester moieties (approximately 70% by weight), mixed with lesser amounts of di-ester phthalates with eight- and ten-carbon alkyl ester moieties (TSCATS Doc. No. #878213843, 1983).

Some DINP products are not only identified by a CAS Registry Number but are also identified as "DINP1," "DINP2," or "DINP3," based upon the manufacturing processes used in their production. Depending on the manufacturing processes used, the quantitative ratio of isomers which make up the final DINP product will vary (see section III, "Production Method"). DINP1 is identified by CAS Registry No. 68515-48-0. DINP2 and DINP3 are both identified by the same CAS Registry No., 28552-12-0. Further information on the various names used to identify DINP by CAS Registry Number can be found in section II, "General Description," of this chapter.

Of the chemicals represented by the four CAS numbers stated above, two (68515-48-0 and 28553-12-0) were reported by industry to EPA under the Chemical Data Reporting regulations at 40 CFR Part 711 of having production volumes of greater than 25,000 pounds per year per manufacturing or importing site. Actual nationally aggregated production volumes for the chemicals represented by these two CAS numbers together ranged in the hundreds of millions of pounds per year. While reviewing data for the hazard assessments, it was noted that only a limited number of the human health and ecological toxicity studies reported the CAS Registry Numbers for the DINP test chemical stocks. When reported, the CAS Registry Numbers were either 68515-48-0 or 28553-12-0. These two CAS Registry Numbers represent the primary DINP products manufactured commercially in the United States. Again, these two CAS Registry Numbers represent a mixture of DINP isomers and not any one single specific DINP isomer. There was no literature available for review which identified a single specific DINP isomer as the test chemical for studies relevant to the evaluation of DINP human health and ecological hazards.

A general summary on DINP including information on the production, use and environmental fate of the chemical is provided below. Because of the close similarity in structure and physical-chemical properties of DINP to other di-ester phthalates, in particular di-2-

ethylhexyl phthalate (DEHP), appropriate environmental fate analogies can be deduced for DINP based upon other di-ester phthalate information. In areas where environmental fate information for DINP is minimal or absent, some fate information presented in this report is based upon other di-ester phthalates. Where information provided is based upon experimental data relevant to other di-ester phthalates, the information is identified as such in this report.

Table 1. provides some physical properties for DINP along with DEHP for comparison since there are considerably more studies on DEHP. DEHP should have higher water solubility, higher vapor pressure, and lower log Kow than DINP since it has one less carbon atom on each of the two branched chains. Estimated physical properties have also been provided for the monoester of DINP because it is anticipated that it will be the major metabolite. As can be seen from the estimated values, the monoester is expected to be more water soluble, less volatile, have a higher Kow, and have a lower Henry's Law constant. Since the pKa for the monoester is 4.32, it is expected that the monoester will exist mostly as the anion in the environment with considerably higher water solubility.

Estimation Programs Interface Suite™ (EPI Suite™ v4.11) is an interface program that through the use of multiple models,generates a model for a chemical's environmental fate based upon physical - chemical properties. Attachments A, B, C, and D provide the EPI Suite™ v4.11-generated physical-chemical properties for the chemicals represented by CAS Registry Numbers 28553-12-0, 71549-78-5, 68515-48-0 and 14103-61-8. The values provided by the EPI Suite database have been gathered from various literature sources, including those derived from published experimental laboratory studies and values generated from theoretical models.

**Table 1. Physical Properties of DINP[a]**

| Phthalate Ester | Water solubility (µg/L) | Vapor pressure (Pa) | Log Kow | Henry's Law constant (HLC)(Pa $m^3$/mol) |
|---|---|---|---|---|
| DINP | 0.6<br>200[c]<br>0.11<br>0.00174[e]<br>0.31[j] | $7.20x10^{-5}$[c]<br>$6.51x10^{-4}$[e]<br>$6.81x10^{-6}$[j] | 8.60[b,j]<br>9.15-9.52[e] | 9.26[b]<br>0.15[d]<br>3.42[f]<br>1.43[g]<br>2.98[h] |
| DINP mono-ester | 472[i] | $4.6x10^{-5}$[i] | 5.22[i] | 0.03[i] |

| | | | | |
|---|---|---|---|---|
| DEHP | 1.9- 400<br>(6 values) | $8.53x10^{-4}$ to $1.31x10^{-5}$<br>(6 values) | 5.11 to 8.35<br>(12 values) | 3.95 |

[a]From Cousins et. al. (2003) except where indicated
[b]Calculated values from Cousins and Mackay (2000) using the 'three solubility' approach
[c]Value used by EPI Suite™ v4.11 to calculate HLC (see also Howard et. al. (1985))
[d]Estimated value from vapor pressure and water solubility - see Attachment A
[e]Estimated values using Suite™ 4.11 - see Attachments A-D
[f]Estimated using the HLC Group Method in EPI Suite™ v4.11 - Attachment B
[g]Estimated using the HLC Group Method in EPI Suite™ v4.11 - Attachment C
[h]Estimated using the HLC Group Method in EPI Suite™ v4.11 - Attachment D
[i]Estimated for undissociated acid (CAS#68515-53-7) using EPI Suite™ v4.11 - Attachment E
[j]Calculated data from Cousins et al. (2003) and Staples et al. (2011).

Because of the strong hydrophobic nature of DINP, studies to accurately measure water solubility have been challenging and have resulted in a wide range of solubility values being reported. The EPI Suite model estimates presented in the attachments to this report state DINP solubility ranges of 0.02 to 0.04 µg/L. An EXXON 1996 study (EXXON, 1996) reported a solubility value of 0.6 µg/L for DINP. Several earlier studies measuring the solubility of DINP reported significantly higher solubility values, some in the mg/L range. The high solubility values reported in some experimental studies could possibly be attributed to the creation of DINP emulsions due to vigorous stirring of the test solution, the entrapment of DINP esters floating on the surface when sub-sampling test solutions, and chemical impurities interfering with the instrumental analytical techniques employed (European Union, 2003). The EXXON 1996 study was designed to eliminate some of the experimental design errors which resulted in the observed elevated solubilities of the earlier studies. This use of the higher water solubility values gave a somewhat lower estimated Henry's Law constants (0.15 Pa $m^3$/mol in Attachment A). Values of 1.43 to 9.26 Pa $m^3$/mol (Table One) are probably more reasonable since they are based on a low water solubility or were calculated directly from structure. Using this range of Henry's Law constant values and assuming no adsorption to suspended solids and sediment, the volatilization half-life from a model river and lake would be 0.6 to 4 days and 14 to 47 days, respectively. However, in most water environments, adsorption will be important and the half- life of volatilization from a pond would be many years (HSDB, 2004) based on the high soil adsorption constant for DINP (Koc = ~ $10^5$ - see Attachments A-D).

## II. General Description

**Appearance:** Colorless, oily liquid

**Molecular Formula:** $C_{26}H_{42}O_4$

**Molecular Weight:** 418.6

**Chemical Class:** Esters

**CAS Registry Number/Synonyms** (ChemIDplus, 2000/CAS, 2000):

**28553-12-0**

| | | |
|---|---|---|
| 1,2-Benzenedicarboxylic acid, diisononyl ester | | Baylectrol 4200 |
| CCRIS 6195 | DINP | DINP 2 |
| Diisononyl phthalate | DINP 3 | Witamol 150 |
| EINECS 249-079-5 | ENJ 2065 | HSDB 4491 |
| Isononyl alcohol, phthalate (2:1) | Vinylcizer 90 | Palatinol DINP |
| Jayflex DINP | Palatinol DN | Palatinol N |
| Phthalic acid, diisononyl ester | Phthalisocizer DINP | |
| Sansocizer DINP | Vestinol NN | Vestinol 9 |

**71549-78-5**

1,2-Benzenedicarboxylic acid, dinonyl ester, branched
Di-(C9-branched alkyl) phthalate
Santicizer 900 (Monsanto)

**68515-48-0**

1,2-Benzenedicarboxylic acid, di-C8-C10-branched alkyl esters, C9-rich
Di(C8-C10) branched alkyl phthalate, DINP 1 (Hellwig)
CCRIS 7927, Di(isononyl) phthalate branched
Diisononyl phthalate, EINECS 271-090-9

**14103-61-8**

Di-(3,5,5-trimethyl hexyl) phthalate
1,2-Benzenedicarboxylic acid, bis(3,5,5-trimethylhexyl) ester
1-Hexanol, 3,5,5-trimethyl-, phthalate
Phthalic acid, bis(3,5,5-trimethylhexyl) ester
Bis(3,5,5-trimethylhexyl) phthalate
Phthalic acid di(3,5,5-trimethylhexyl) ester

## III. Production Method

The production of diisononyl phthalates involves the reaction of phthalic anhydride and alcohols. The isomeric composition (degree of branching in the alkyl moiety) found in a DINP isomer is dependent upon the process used to synthesize the alcohols used in its production. For

example, the alcohols used in the synthesis of DINP 2 (CAS Registry No. 28553-12-0) are derived from the dimerization of n-butene followed by carbonylation and hydrogenation processes which results in 95% of the main alcohol components as alkyl substituted octanols or heptanols (Hellwig, 1997). The alcohols used in the synthesis of DINP 3 (also CAS Registry No. 28553-12-0) are derived by the dimerization of n-butene and iso-butene resulting in at least 60% of the main alcohol component as alkyl substituted hexanols (Hellwig, 1997). The alcohols used in the production of DINP 1 (CAS Registry No. 68515-48-0) are manufactured by polymerization of propylene and/or butene followed by carbonylation and hydrogenation processes (EXXON, 2000 and Kirk-Othmer, 1996). The resulting alcohols are a mixture of eight, nine, and ten carbon alcohols. The nine-carbon alcohol fraction consists of roughly equivalent amounts of 3,4-, 4,6-, 3,6-, 4,5- and 5,6-dimethyl-heptanol-1 (Hellwig, 1997). Reflecting the mixed nature of the alcohols used in its production, DINP-1 consists of a mixture of di-ester phthalates with alkyl ester moieties consisting of eight, nine and ten carbons at approximate percentages of five, seventy-five and twenty, respectively (TSCATS Doc# 878213843, 1983).

Once the desired alcohol feedstocks have been obtained, the first stage in the production of diisononyl phthalate is the reaction of phthalic anhydride with the synthesized alcohols (alcoholysis). This stage results in the production of the monoester. The second stage is the conversion of the monoester to a diester by further reaction with the alcohol. This esterification process requires the use of acid, titanates or tin (II) catalysts (Elvers et al. 1992).

## IV. Use Properties

Diisononyl phthalates constitute a family of di-ester phthalates widely used as plasticizers, increasing the softness and flexibility of polymeric materials (Kirk-Othmer, 1996). Approximately 95% of the DINP produced is used in PVC applications (European Union, 2003). Usually phthalate ester plasticizers, stabilizers, lubricants, and pigments are added to PVC to be compounded, and the mixture is subsequently processed at elevated temperatures into a fabricated product. It has been theorized that at elevated temperatures, phthalate esters migrate into PVC resins displacing the polar linkages between the PVC resin chains and establishing their own polar linkages within the PVC (Kirk-Othmer, 1996). This displacement by phthalate esters results in an increase in the free volume which provides PVC with greater flexibility and

softness.

For phthalate esters the amount of a plasticizer required to impart a specified softness to PVC increases as the length of the alkyl carbon chain increases. For example, DINP will perform less efficiently as a plasticizer than di-butyl phthalate (Kirk-Othmer, 1996). The performance of a PVC product at elevated temperatures is directly related to the volatility and, in turn, the molecular weight of the plasticizer used as part of its fabrication. Higher molecular weight phthalate esters provide greater thermal stability to the final PVC product. The decrease in volatility of higher molecular weight phthalates like DINP, versus di-butyl phthalate, reduces the loss of the plasticizer (DINP) during the processing of the final PVC product as well as the loss of the plasticizer during the use of the product at elevated temperatures (Kirk-Othner, 1996).

## V. Occurrence

Phthalate plasticizers are not covalently bound to plastics and will slowly leach or volatilize from plastics. Phthalates can also be extracted from the plastic. Esters which are of higher molecular weight and/or have a greater degree of alkyl branching demonstrate slower extraction rates (Kirk-Othmer, 1996). Solvents vary in their effectiveness in extracting phthalate esters from PVC. Light weight organic solvents are the most effective, followed by oils and then water. Phthalate levels in freshwaters have been measured at concentrations from nondetectable to 10 µg/l (Kirk-Othmer, 1996). As a plasticizer, DINP has been commonly used in the production of children's toys. Studies by the Canadian Government have shown DINP to be present in the range of 3.9 to 44% by weight in 64% of all of children's products tested (Health Canada, 1998). A study by the U.S. Government (CPSC, 1998) detected DINP in 31 of 35 children's products tested which were composed of PVC. The DINP levels ranged from 15 to 54 percent.

There is a very limited amount of environmental monitoring data on DINP. However, in the EU Risk Assessment Report on DINP (European Union, 2003) the large amount of DEHP monitoring data was compared to the limited amount of DINP. It was noted that the quantities of DEHP used in Western Europe are about twice the amount of DINP used. The EU report suggested that the environmental concentrations of DINP are of the "same order of magnitude or lower than those of DEHP." Reported DINP concentrations were: Surface water - <0.1 to 1.09 µg/L; sediment - <25 to 6161 (median in one study 161) µg/kg dw; Mussels - < 500 to 810 µg/kg

dw; Algae - < 100 µg/kg dw; Sewage Sludge - 110 to 23,000 µg/kg dw; and Soil - 1 to 910 µg/kg dw (European Union, 2003).

## VI. Environmental Fate

### A. Abiotic Degradation

1. Hydrolysis

Abiotic hydrolysis is not considered a major mechanism for the degradation of phthalate esters under typical environmental conditions (Staples et al. 1997). The hydrolysis of DINP can be characterized as a two-step hydrolytic process, with the first step resulting in the creation of a monoester phthalate and one free nine-carbon alcohol molecule, and the second hydrolytic step resulting in the formation of phthalic acid and the creation of a second nine- carbon alcohol. As the length of the alkyl chain increases, the phthalate hydrolysis half-life increases (Wolfe, 1980a). At pH 8 and 30° C, the half-life for di-ethyl hexyl phthalate (DEHP) has been calculated to be more than 100 years (Wolfe, 1980a). Under acidic conditions (pH 4- 6), the hydrolysis rate of phthalate esters is estimated to be four orders of magnitude slower as compared to alkaline (pH 8-10) hydrolysis rates (Staples et al. 1997).

2. Photodegradation

The photodegradation of phthalate esters in the surface of sunlit waters does not appear to be a significant contributing process to the environmental degradation of phthalates esters. No experimental data could be found relating to direct photolysis of phthalate esters. Wolfe et al. (1980b), using models employing linear free energy relationships for selected phthalates esters (including di-n-octyl phthalate (DNOP) and di-ethyl-hexyl phthalate (DEHP)), estimated the total phthalate load reduction due to proteolysis to be between 0.0 and 2.0 percent for river, pond, and eutrophic lake systems and 4.6 to 13.9 percent for oligotrophic lake systems. Based on the data of Wolfe et al. (1980b), it is estimated that aqueous photo-oxidation half-lives range from 2.4 to 12 years for di-ethyl phthalate and di-n-butyl phthalate and range from 0.12 to 1.5 years for DEHP (Staples et al. 1997).

In contrast to aqueous photodegradation, atmospheric photodegradation of phthalate

esters occurs at a significantly faster rate, although it must be kept in perspective that the available mass of a phthalate ester in the atmosphere is limited by the very low volatility of the phthalate ester. Wolfe et al.(1980b) using the EXAMS model for selected phthalate esters estimated the total phthalate load reduction due to volatilization at steady-state from an aqueous environment to be 2 and 3 percent for DEHP in pond, eutrophic lake and oligotrophic lake systems. For DNOP the estimates range from 16 to 24 percent for the same water system models. Using a river system model, the percent mass of phthalate reduction attributed to volatilization was estimated at zero percent for both DNOP and DEHP.

Reaction with hydroxy radicals is considered the most important photodegradation process for organic chemicals (Meylan, 1993). Model estimates indicate a half-life value ranging from five to twelve hours. One study reports the experimental half-life for DEHP of approximately one day (Staples et al. 1997). The results of models indicate that as the phthalate ester alkyl chain length increases, the potential of phthalate esters to photo-oxidize increases (Meylan, 1993).

**B. Biotic Degradation**

Limited biodegradation data from aerobic and anaerobic studies that simulate activated sludge conditions and freshwater sediment conditions are available for DINP. Due to their physical-chemical properties, phthalate esters have an affinity for depositing onto soil and sediments. Wolfe et al. (1980b) indicated in his models for aqueous systems for DNOP and DEHP, that 70 to 98 percent of all phthalates would reside in the bottom sediment fraction of river, ponds and eutrophic lakes.

Staples et al. (1997) provides a summary of numerous phthalate ester biodegradation studies in aerobic and anaerobic soil, sludge and sediment environments. DINP studies conducted using activated sludge under aerobic conditions indicate degradation between57 and 71 percent and primary degradation of greater than 90 percent after 28 days at 22° C.Studies conducted by Scholz et al. (1997) reported 79 percent biodegradation of DINP in studies using activated sludge under aerobic conditions after 28 days at 22° C. In contrast, Shelton et al. (1984) reported less than 25% biodegradation of DNOP and DEHP in sludge under anaerobic conditions after 10 weeks at 35° C. Johnson and Heitkamp (1984) experimentally found the primary degradation for both DINP and diisooctyl phthalate (as indicated by the formation of

$^{14}CO_2$ from the carbonyl carbon) in freshwater sediment under aerobic conditions to be less than 5% after 28 days at 22° C. The observed biodegradation rate for dibutyl phthalate was significantly faster with 85% primary degradation after 28 days.

Johnson and Heitkamp concluded that the rate of biodegradation of phthalate esters has an inverse relationship to the length and branching associated with their alkyl chains. The phthalate esters containing longer chain, more branched alkyl esters moieties, like DINP, are more persistent in freshwater sediment environments than phthalate esters containing short-chained minimally branched alkyl ester moieties.

Staples et al. (1997) formulated three basic generalizations concerning the anaerobic biodegradation of phthalates esters: 1) The rate of degradation under anaerobic conditions is delayed compared to aerobic conditions; 2) Alkyl chain length can impact the extent of anaerobic degradation; and 3) The bacterium used can influence degradation rates. A review by the Danish Environmental Protection Agency indicated that DINP demonstrates high primary biodegradation in simulated sewage treatment plants but very low rates of primary biodegradation in sediment-water systems under both aerobic and anaerobic conditions (Danish Environmental Protection Agency, 1999). The review also indicates that DINP appears to be efficiently removed from wastewater as a part of the sludge fraction during sewage treatment plant processing; however, limited degradation of the DINP was observed in the sludge fraction based upon the mass balance of DINP entering the sewage treatment plant in the wastewater and leaving as a part of the sludge fraction.

Staples et al. (1997) summarized the biotic degradation mechanism of phthalate esters from several research studies. The microbial metabolism of phthalates under both aerobic and anaerobic conditions begins by ester hydrolysis resulting in the formation of the monoester and the corresponding alcohol. The next step of enzymatic degradation results in the formation of phthalic acid and another corresponding alcohol. Under aerobic conditions the phthalic acid undergoes hydrolysis forming 3,4- or 4,5- dihydroxy phthalate which breaks down to form protochatechuate (dihydroxybenzoic acid). Aromatic ring cleavage can then occur via either an ortho pathway resulting in the formation of pyruvate and oxaloacetate or a meta pathway yielding a beta-ketoadipate that is further degraded to acetyl CoA and succinate. Under biotic anaerobic conditions, the phthalic acid degradation product is broken down by the same pathway used for benzoate.

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# Chapter 2
# Human Health Assessment

## I. Executive Summary

Currently available data on the carcinogenicity, reproductive, developmental, or chronic toxicity associated with diisononylphthalate (DINP) exposure is from studies in rodents with few studies in higher organisms from rabbits to primates. The only studies available in humans pertain to the metabolism of DINP and do not examine effects resulting from exposure. The available DINP data in primates includes a 14-day oral study in cynomolgus monkeys (Pugh et al. 2000) and a 90-day oral study in marmosets (Hall et al. 1999). Primate studies were insufficient to evaluate DINP for carcinogenicity as well as for potential reproductive and developmental effects. Therefore, the evaluation of the chronic, reproductive, and developmental toxicity endpoints for DINP relied predominantly upon oral exposure studies conducted in rodents. There are no reliable inhalation toxicity studies available, most likely because the low vapor pressure of DINP usually precludes inhalation of any significant amount, except perhaps as an aerosol adsorbed to airborne particulates.

The reproductive and developmental toxicity of DINP has been characterized in one- and two-generation reproductive toxicity studies (Waterman et al. 2000); in two prenatal developmental toxicity studies (Hellwig et al. 1997; Waterman et al. 1999); and in single- and multi-dose perinatal exposure studies in rats (Gray et al. 2000; Masutomi et al. 2003). The reproductive and developmental toxicity of DINP has not been studied in rabbits or primates. Additional research by Clewell et al. (2011a and 2011b) characterized the potential developmental and reproductive effects of DINP administered during pregnancy, gestation, and lactation on the sexual development of the male fetus in rats. There is a paucity of multi-dose studies addressing the antiandrogenic effects of DINP on the development of the male reproductive system. The need for examination of this effect is indicated from the results of Gray et al. (2000) who observed malformations of the male reproductive system in offspring of rats perinatally exposed to 750 mg/kg/day and Boberg et al. (2001) who observed permanent reproductive malformations in male rats after *in utero* exposure to doses ≥600 mg/kg/day DINP. In a neurodevelopmental study (Lee et al. 2006), maternal rats exposed to DINP and re-exposed after maturation showed changes at the mRNA level in genes involved

in sexual development with a lowest observed effect level (LOEL) of 2 mg/kg/day. Several short-term studies have clearly shown the effects of DINP exposure on the male reproductive system at the hormone, protein, and genomic levels (Boberg et al. 2011; Hannas et al. 2011; Clewell 2013, 2011a, 2011b; Furr et al. 2014).

Observed developmental effects in rats include decreased body weight of pups during lactation and adverse skeletal and kidney effects, which occurred in the absence of maternal toxicity. There were statistically significant pup body weight decrements with the lowest observed adverse effect level (LOAEL) of 143–285 mg/kg/day (during gestation and lactation) in male and female pups and in both F1 and F2 generations of the two-generation reproductive toxicity study (Waterman et al. 2000). In addition, significant decrements in postnatal pup weights were also noted in a one-generation reproductive toxicity study (Waterman et al. 2000) and in a multi-dose perinatal exposure study (Masutomi et al. 2003). The two developmental toxicity studies that evaluated fetal malformations and variations also had consistent results. In both studies, DINP exposure resulted in increased incidence of rudimentary lumbar and supernumerary cervical ribs as well as adverse renal effects in fetuses; the effective doses were similar for those effects (Hellwig et al. 1997; Waterman et al. 1999). There was a dose-related increase in the incidence of litters with rudimentary lumbar ribs at 500 mg/kg/day and a statistically significant increase in the incidence of supernumerary cervical ribs at 1,000 mg/kg/day. A dose-related increase in the mean percent of pups in a litter with dilated renal pelvises was also reported, with the response at the highest dose (1,000 mg/kg/day) being statistically significant. The percentage of fetuses with dilated renal pelvises was significantly increased at all treatment levels (i.e., 100, 500, and 1000 mg/kg/day).

No effects have been noted in the male or female reproductive system based upon the results of one- and two-generation reproductive toxicity studies that evaluated reproductive performance and histological effects of DINP on the gonads (Waterman et al. 2000). However, endpoints indicative of *in utero* androgenic activity were not examined in these studies.

Evidence for antiandrogenic effects of DINP has been observed in several studies. Perinatal exposure of pregnant Sprague-Dawley rats to 750 mg/kg/day DINP by gavage in

corn oil on gestation day 14 through postnatal day 3 resulted in developmental effects in male offspring (areolas and retained nipples, testicular abnormalities) that were consistent with an antiandrogenic mode of action (MOA) (Gray et al. 2000; Boberg et al. 2011). Dosing of pregnant Wistar rats with 750 mg/kg/day DINP in peanut oil during gestation significantly reduced fetal testicular testosterone content and *ex vivo* production of testosterone from the fetal testis, suggesting that the antiandrogenic effects of DINP result from disruption of steroidogenesis in the fetal testis (Borch et al. 2004). Similarly, dosing pregnant SD rats with 500 to 1500 mg/kg/day DINP on GDs 14-18 resulted in dose-dependent reductions in fetal testis testosterone production and reduced expression of genes involved in cholesterol transport and hormone synthesis *(StAR, Cyp11a, CYP11b1, Cyp11b2, Cyp17a1, Hsd3b, Scarb1*), which is consistent with the observed reduction in testosterone production (Hannas et al. 2011; Gray et al. 2021). These data are supported by more detailed mechanistic studies on a structurally related compound, di(*n*-butyl) phthalate, which showed rapid and reversible decreases in several proteins required for cholesterol transport and steroidogenesis in the fetal testis following exposure of pregnant dams to 500 mg/kg/day during gestation (Thompson et al. 2004).

DINP exposure is linked to a variety of non-apical end points ranging from (1) increased blood pressure (Deng et al. 2019), (2) epigenetic modifications, (3) genes associated with sex-specific traits (Neier et al. 2019), and (4) regulation of bone density (Hwang et al. 2017). DINP exposure has been recently linked to atopic dermatitis, though DINP itself is not a sensitizer, exposure could aggravate a previous dermal stimulus (Imai et al. 2006; Koike et al. 2010; Sadakane et al. 2014; Wu et al. 2015; Kang et al. 2016). DINP exposure has also been linked to brain injury leading to cognitive deficits (Ma et al. 2015, Peng et al. 2015).

An independent perinatal exposure study detected histological effects in the testes and ovaries of offspring of female Sprague-Dawley rats treated with a maternally toxic dietary dose of DINP (1165–2656 mg/kg/day) from gestation day 15 through postnatal day 10 (Masutomi et al. 2003).

According to EPA Developmental Toxicity Guidelines (U.S. EPA 1991a), a change in offspring body weight is a sensitive indicator of developmental toxicity. Significant decreases in body weight in young animals can be associated with developmental delays and lifelong

mental and physical deficiencies (U.S. EPA 1991a; Hood 1996). The biological significance of an altered incidence of anatomical variations is difficult to assess, but several factors can be considered such as knowledge about developmental stage (e.g.,with skeletal ossification), background incidence of certain variations (e.g., accessory ribs), absence of maternal toxicity, or other strain- or species-specific factors. In the case of DINP, variations were significantly increased in a dose-related manner; therefore, these effects are considered as an indication of developmental toxicity. Although the effect on lumbar ribs was more pronounced, the effect on cervical ribs is of greater toxicological concern. Cervical ribs are seen infrequently in controls and their presence may indicate a disruption of gene expression. While some investigators have suggested that occurrence of dilated renal pelvises represents a transient developmental delay (Woo and Hoar 1972), other researchers have suggested that developmental delays can persist well into postnatal life and that physiological function is compromised in the affected animals (Kavlock et al. 1987, 1988).

DINP has been tested in several carcinogenicity studies in rats and mice. Statistically significant increases in many tumor types have been observed, such as increases in hepatocellular tumors, MNCL of the spleen, and renal tubular cell carcinomas. In addition, other tumor types considered rare and/or uncommon have been noted in DINP-treated animals, including renal transitional cell carcinoma, pancreatic islet cell carcinoma, testicular interstitial (Leydig) cell carcinoma, and uterine adenocarcinoma. There are multiple lines of evidence that DINP presents a cancer hazard, but a complete cancer risk determination, including exposure assessment, is currently being considered as part of a manufacturer-requested risk evaluation under TSCA.

DINP produced chronic liver toxicity in rats. Two chronic oral exposure studies of DINP reported dose-related increases in the incidence of spongiosis hepatis (a degenerative lesion of the liver) in treated male rats (Lington et al. 1997; Moore 1998a). The LOAELs for spongiosis hepatis in these studies were 152 mg/kg/day and 359 mg/kg/day, respectively. The apparent qualitative difference in spongiosis hepatis in the two rat studies may reflect differences in the range of doses tested. Dose-related increases in liver and kidney weights in both male and female rats were also reported in both studies at LOAELs of 152 mg/kg/day (males) and 184 mg/kg/day (females), respectively, while male rats (at 152 mg/kg/day) had increased serum levels of alkaline phosphatase and transaminases (indicative of liver cell

damage). Liver slides from the two chronic rat studies were reviewed by the Pathology Working Group, an expert panel formed to review the critical pathology reported in these studies. That review confirmed that the chronic liver lesion spongiosis hepatis was increased in male rats in both chronic rodent studies (EPL 1999). Although spongiosis hepatis occurred in perisinusoidal cells, it was believed to be unrelated to peroxisome proliferation-induced cancer that occurs in hepatocytes (see more below).

DINP produced liver tumors in rats and mice following chronic exposure (Lington et al. 1997; Moore 1998a). Statistically significant increases in the incidence of liver tumors in rats and mice exposed to DINP were reported in two independent chronic oral exposure studies. The MOA for induction of hepatic tumors by DINP might involve peroxisome proliferation. Peroxisome proliferators are a structurally diverse group of non-mutagenic chemicals that interact variably with peroxisome proliferator activated receptors (PPAR) to elicit characteristic adaptive responses in the liver. DINP is classifiable as a hepatic peroxisome proliferator based on characteristic signs of increased liver weight, microscopic evidence of peroxisome proliferation, and induction of marker enzymes. Currently available evidence indicates that the PPAR alpha isoform mediates induction of the pleiotropic response associated with peroxisome proliferation in rodents (Klaunig et al. 2003). Studies in PPAR alpha-deficient mice confirm that this receptor subtype mediates physiological and biochemical hepatic responses associated with peroxisome proliferation in animals treated with DINP—including increases in liver weight (in young PPAR alpha-deficient mice), hepatocyte proliferation, palmitoyl CoA oxidase activity, and levels of enzymes associated with beta- and omega-oxidation of fatty acids (Valles et al. 2003). In rats and mice, interaction with PPAR alpha is believed to be the precursor for development of liver tumors.

Recent scientific data suggest that liver tumors developing in rats and mice chronically exposed to phthalates are mechanistically related to PPAR alpha activation (Valles et al. 2003). Recombinant Human PPAR alpha was shown to be functionally equal to mouse recombinant PPAR alpha in mice lacking functional PPAR alpha (i.e., PPAR alpha null mice) in inducing peroxisome proliferator pleiotropic response (Yu et al. 2001). Some researchers believe that the data are sufficient to establish that liver tumors in rodents treated with DINP are likely a result of a PPAR alpha agonist MOA (e.g., Kaufmann et al. 2002). Research developments in the areas of peroxisome proliferation and PPAR alpha agonism

have prompted additional evaluation of the human relevance of rodent tumors induced by PPAR alpha agonists (e.g., Melnick et al. 2001; Klaunig et al. 2003; Felter 2018). On the other hand, the metabolites of DEHP and DINP have shown to be equally potent in activating hCAR2, suggesting multiple MOAs for non-genotoxic carcinogenesis (Laurenzana et al. 2016).

In two chronic oral studies using Fischer (F-344) rats (Lington et al. 1997; Moore 1998a), there were clear, statistically significant increases in the incidences of mononuclear cell leukemia (MNCL). MNCL is life threatening in F-344 rats and results in a decreased life span. Although MNCL is recognized as a common neoplasm in Fischer rats, the MOA for MNCL induction is not completely understood. In addition, there are differing views on the existence of a close human correlate to MNCL (Caldwell 1999; CHAP 2001). Some researchers have suggested that based on the biological and functional features in the F-344 rat, MNCL is analogous to large granular cell leukemia (LGL) in humans (e.g., Reynolds and Foon 1984). Therefore, the significance of MNCL and its biological relevance for human cancer risk remains uncertain and cannot be discounted. EPA believes that the available data are inadequate for delineation of a plausible sequence of events leading to development of MNCL in rats exposed to DINP. This lack of information precludes an assessment of the relevance of DINP effects on occurrence of MNCL to humans.

The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency (CalEPA) has published a document on the evidence on the carcinogenicity of DINP. In that report, members of the Carcinogen Identification Committee (CIC) concluded that DINP has been clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer and should be listed under Proposition 65 as a carcinogen (OEHHA 2013a). Accordingly, DINP was listed under Proposition 65 at the end of 2013. DINP has not been classified for its potential carcinogenicity by international agencies.

Bacterial and *in vitro* mammalian gene mutation assays, with or without metabolic activation, have consistently shown that DINP is not mutagenic. DINP has also been evaluated in both *in vivo* and *in vitro* cytogenetic assays, with results consistently supporting a finding that DINP is not genotoxic.

Kidney tumors were noted in male rats that were chronically exposed to 733 mg/kg/day

DINP by the oral route (Moore 1998a). EPA believes these kidney tumors are associated with a male rat-specific mechanism involving alpha-2u-globulin accumulation in the kidney, and that this mechanism is not appropriate for estimating hazard in humans. This conclusion is based on the fact that all three EPA criteria for the alpha-2u-globulin MOA were met; to date, EPA has not found other information or data to suggest that another mechanism is likely to be involved.

The kidney is also a non-cancer target organ for DINP. Kidney changes in female mice (increased severity in nephrotoxicity) (Moore 1998b) as well as male and female rats (increased kidney weights, compromised ability to concentrate urine) (Lington et al. 1997; Moore 1998a) are indicative of kidney toxicity.

## II. Toxicokinetics

An overview of data available for the oral and dermal toxicokinetics of DINP is provided as background information for the hazard assessment.

### a) Oral Administration

McKee et al. (2002) examined the metabolism of DINP (CASRN 68515-48-0) in male and female F344 rats. All rats administered single oral doses of 50 or 500 mg/kg [$^{14}$C]DINP appeared healthy and without significant toxicity. No differences in excretion were apparent in either sex at either dose. At the low dose, 50% of the radioactivity was recovered in the urine and the remainder in the feces. At the high dose, 35–40% of the administered dose (radioactivity) was excreted in the urine and the remainder in the feces, suggesting an inverse relationship between dosage and absorption. In repeated dose studies, rats were administered 50, 150, and 500 mg/kg/day [$^{14}$C]DINP for 5 days and excretion was evaluated. Fifty to sixty percent of the administered material was excreted in the urine. In the repeated dose studies about 60% of the administered dose was excreted at all doses, suggesting an elevation of esterase activity and more rapid conversion to monoester following repeated treatment. The elimination (half-life) of absorbed [$^{14}$C]DINP was about 7 hours. Tissue distribution patterns of DINP revealed that absorption from the gastrointestinal (GI) tract was rapid after both acute and repeated dosing. DINP translocated from the GI tract via the blood rapidly to liver and kidney. DINP is mainly hydrolyzed in the GI tract after oral administration. The metabolic profile suggests that DINP is recovered mainly as oxidized products and phthalic

acid and very little as the parent or the metabolite mono-isononyl phthalate (MINP), suggesting that DINP is rapidly metabolized in the GI tract to the corresponding monoester with a second hydrolysis step in liver to phthalic acid. A summary of DINP absorption in various species is presented in [ REF _Ref99634379 \h * MERGEFORMAT ].

In a dermal study, three groups of rats were exposed to [$^{14}$C]DINP at 50 mg/kg/day as "conditioned skin," "non-conditioned skin," and "occluded." A total of 2–4% of dermally administered DINP was absorbed in 7 days with an absorption rate of approximately 0.3–0.6% per day. The metabolic profile of dermal absorbed DINP was similar to DINP metabolic profile from oral administration.

**Table [ SEQ Table * ARABIC ]. Absorption and Excretion Summary of DINP**

| Species | Dose | Source | Absorption | Reference |
|---|---|---|---|---|
| Human | 1.28 mg/kg | Urine | 44% over 48 h | Koch and Angerer, 2007 |
| Human | 0.78 and 7.3 mg | Urine | 33 ± 6.4% over 48 h | Anderson et al. 2011 |
| Rat | 50 mg/kg<br>500 mg/kg<br>50–500 mg/kg<br><br>50, 150, or 500 mg/kg/day for 5 days | Urine<br>Urine<br>Estimated urine + bile<br><br>Urine<br>Estimated urine + bile | 49% over 72 h<br>39% over 72 h<br>75% over 72 h<br><br>56–62% over 24 h, 62–64% over 72 h<br>90% over 72 h | McKee et al. 2002 |
| Rats (non-pregnant) | Single dose of 300 mg/kg | Urine | Mono(carboxy-isooctyl)phthalate (MCIOP) 0.042%<br>oxidative metabolites 0.051% | Silva et al. 2006 |

Clewell et al. (2013) also observed a similar trend in the absorption pattern in rats. The percentage of absorbed dose of DINP decreased at higher doses of 750 mg/kg bw/day compared to 250 mg/kg bw/day in rats administered DINP via gavage from gestation day (GD) 12 to 19.

One of the few studies designed to understand the metabolism of phthalates in humans, a male volunteer aged 63 was given a single oral dose of 1.27 mg of deuterium-labeled

DINP-2/kg-bw. DINP was found to be rapidly distributed and eliminated in humans, similar to rats (Koch and Angerer 2007). The postulated metabolic pathway of DINP in humans is summarized in [ REF _Ref99698295 \h * MERGEFORMAT ]. Approximately 44% of the administered dose was recovered in urine over 48 hours in the form of the following metabolites: (1) 20.2% as OH-MINP (MHINP; based on measured standard of 7OH-MMeOP); (2) 10.7% as carboxy-MINP (MCIOP; based on measured standard of 7-carboxy-MMeHP); (3) 10.6% as oxo-MINP (MOINP; based on measured standard of 7oxo -MMeOP); and (4) only 2.2% as MINP (Koch and Angerer 2007).

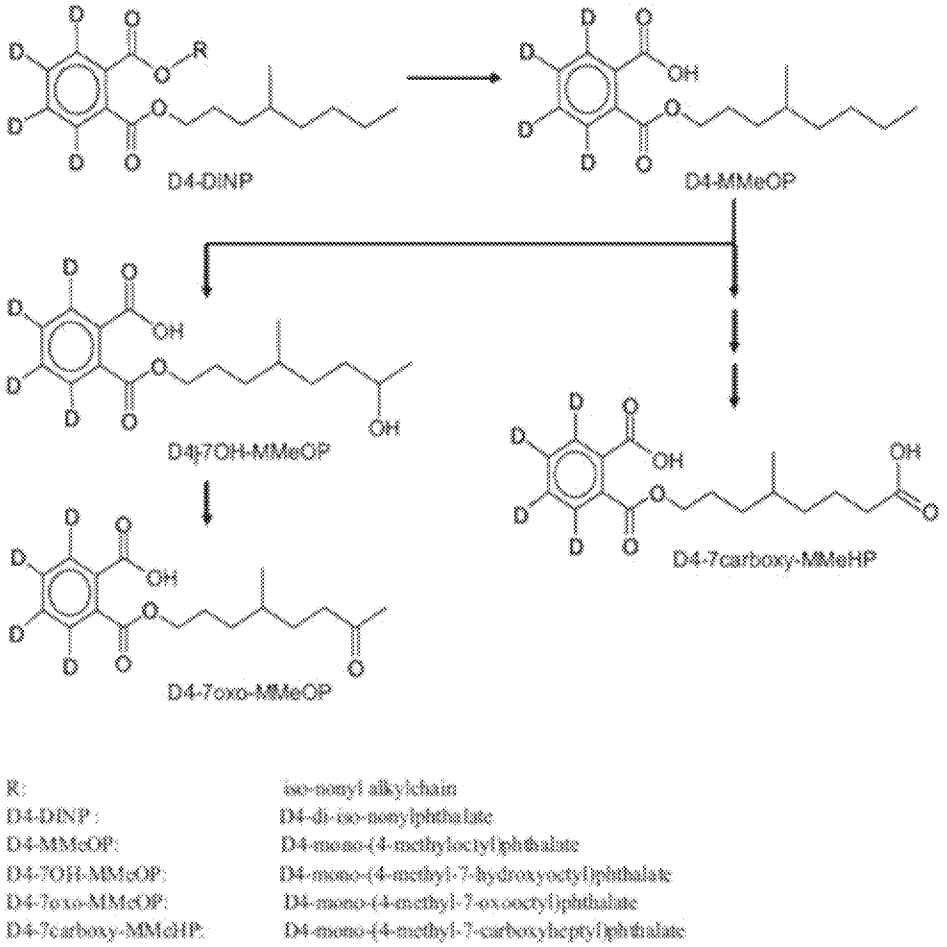


**Figure** [ SEQ Figure * ARABIC ]. **Postulated DINP Metabolism in Humans (Figure from Koch and Angerer, 2007).**

In a second human study, Anderson et al. (2011) studied the kinetics of DINP (labeled with deuterium) in 10 adult male (20–42 years of age) and 10 female (18–77 years of age) volunteers after a single oral exposure. Two dose levels were administered—a low dose of

0.78 mg (0.010 mg/kg-bw for males; 0.011 mg/kg-bw for females) and a high dose of 7.3 mg (0.090 mg/kg-bw for males; 0.107 mg/kg-bw for females). The total radioactivity recovered from the previously identified metabolites combined was 33±6.4% of the labeled DINP in urine over 48 hours. Metabolite half-lives were estimated to be 4–8 hours with over 90% excreted in the first 24 hours of urine collection (Anderson et al. 2011).

Silva et al. (2006) characterized the different ω- and ω-1-oxidation oxidative metabolites found in urine after administration of a single dose of 300 mg DINP/kg-bw to non-pregnant Sprague-Dawley rats. MCIOP was the major urinary metabolite recovered, while MINP and DINP were not found in significant amounts in the urine (Silva et al. 2006). Clewell et al. (2013) determined that MCIOP was also the most abundant metabolite (76–81% of the urine metabolites) in rats exposed via gavage to up to 750 mg DINP/kg-bw per day during GD 12–19, while MINP and its glucuronidated form (MINP-Gluc) were almost negligible. Because these metabolites were localized in maternal plasma, and MINP was present at similar concentrations as MCIOP, it was suggested that (1) urinary clearance of both MINP and MINP-Gluc is limited, and (2) these metabolites were poor predictors of plasma and tissue disposition for DINP (Clewell et al. 2013).

Clewell et al. (2013) also characterized the metabolite disposition of DINP in the fetus, and it revealed that MINP and its oxidative metabolites along with its glucuronidated form were all present in the fetal plasma, testes, and amniotic fluid. MINP-Gluc was present at higher concentrations in the fetal plasma than the maternal plasma (in contradiction with what was observed with the other metabolites), indicating potential placental transfer of this MINP-Gluc or, more likely, that conjugation could occur in the fetus by fetal phase II detoxification enzyme systems (Clewell et al. 2013). A summary of different metabolites found in human and rat urine after oral administration of DINP is presented in [ REF _Ref99634499 \h * MERGEFORMAT ].

| **Table [ SEQ Table * ARABIC ]. Metabolites of DINP Identified in Urine from Rats and Humans after Oral Administration** | | |
|---|---|---|
| **Metabolites** | **Abbreviation** | **Reference (Species)** |
| Monoisobutyl phthalate | MINP | Anderson et al. 2011 (human)<br>Suzuki et al. 2012 (human)<br>Koch and Angerer 2007 (human)<br>Calafat et al. 2006 (rat) |
| Glucuronidated MINP | MINP-Gluc | Clewell et al. 2013 (rat) |
| [mono-(4-methyl-7-carboxyheptyl) phthalate] representing:<br>Mono(carboxyisooctyl) phthalate | [D4-7carboxy-MMeHP]<br>CO2-MINP;<br>MCIOP | Anderson et al. 2011 (human)<br>Koch and Angerer, 2007 (human) |
| [D4-mono-(4-methyl-7-hydroxyoctyl) phthalate] representing:<br>Mono(hydroxyisononyl) phthalate | [7OH-MMeOP]<br>for OH-MINP;<br>MHINP | Anderson et al. 2011 (human)<br>Schulz et al. 2012 (human)<br>Koch and Angerer 2007 (human)<br>Silva et al. 2006 (rat) |
| [D4-mono-(4-methyl-7-oxooctyl)phthalate] representing:<br>Mono(oxoisononyl) phthalate | [7oxo-MMeOP]<br>for Oxo-MINP;<br>MOINP | Anderson et al. 2011 (human)<br>Schulz et al. 2012 (human)<br>Koch and Angerer 2007 (human)<br>Silva et al. 2006 (rat) |
| Monocarboxylisononyl phthalate | cx-MINP | Schulz et al. 2012 (human) |
| Mono-carboxy-isooctyl phthalate | MCIOP | Silva et al. 2006 (rat) |
| Mono(carboxy-isoheptyl) phthalate | MCiHpP | Silva et al. 2006 (rat) |
| Mono-(3-carboxypropyl) phthalate | MCPP | Calafat et al. 2006 (rat) |
| Mono-n-octyl phthalate | MnOP | Calafat et al. 2006 (rat) |
| Phthalic Acid | PA | McKee et al. 2002 (rat) |

b) Dermal Administration

Data obtained from *in vivo* and *in vitro* studies have shown that absorption of phthalates through rat and human skin decreases as the length of the alkyl chain increases (Scott et al. 1987; Elsisi et al. 1989; Mint and Hotchkiss 1993; Mint et al. 1994).

DINP, like all phthalates, is not absorbed well through skin. McKee et al. (2002) exposed rats dermally to [$^{14}$C]DINP and radioactivity recovered from the application site was greater than or equal to 92%. Dermal absorption was estimated to be 2–4% over 7 days based

on amount of applied-dose recovered in urine, feces, and tissues. Further, the study showed that radioactivity increased with time in skin (0.12, 0.26, and 0.27% of the applied dose following 1, 3, and 7 days of exposure, respectively). After 7 days of exposure, the distribution of radiolabeled DINP in other tissues was as follows: GI tract (0.097%), fat (0.053%), muscle (0.024%), and other organs (≤0.009%). The fractions of applied dose recovered in tissues were higher at a lower dose. However, the total percent absorbed at the low dose (3%) did not differ significantly from the high dose (4%), in contrast to the oral absorption, indicating the absence of the overloading effect on the "mismeasure of dermal absorption," as described by Kissel (2011).

Based on this study, it is expected that no more than 4% is dermally absorbed in rats. Although there is no data on the dermal absorption of DINP in humans, it was recognized by several organizations (i.e., Danish EPA, European Chemicals Agency [ECHA], and National Industrial Chemicals Notification and Assessment Scheme [NICNAS]) that absorption of phthalates is lower in human skin than rat skin. This is specifically based on data from *in vitro* migration studies conducted with DEHP and other phthalates (Scott et al. 1987; Barber et al. 1992; Mint and Hotchkiss 1993). Therefore, it is also considered appropriate to consider a dermal absorption of 4% for humans. Similar values were selected by NICNAS and ECHA in their assessment report on DINP (NICNAS 2008; ECHA 2013a).

c) Inhalation Absorption

Currently, there is no information available to determine the absorption of DINP through lungs. However, the available acute inhalation toxicity study indicates that there is not significant acute toxicity associated with DINP following inhalation exposure. An available repeated dose study for DIDP as a read across to DINP indicated no significant toxicity associated via the inhalation route (ECHA Dossier).

d) Conclusions on Toxicokinetics

The toxicokinetic data indicate that a single low acute oral dose (50 mg/kg) of radiolabeled DINP administered to rats was readily absorbed (50%); however, in a high dose (500 mg/kg) only about 40% was recovered, suggesting an inverse relationship between dose administered orally and recovered in urine. Notably, following repeated dose administration,

the percentage of DINP recovery increased to about 60%, suggesting that "priming" of the system improves the absorption and elimination of DINP. Orally administered DINP is rapidly metabolized in the gut to MINP and distributed via blood to major tissues, particularly the liver. DINP metabolites were excreted in urine and to a lesser extent in feces. DINP was de-esterified to the monoester MINP in the gastrointestinal (GI) tract, which was further metabolized by side-chain oxidation of the ester group or by hydrolysis to phthalic acid. The formation of oxidation products appeared to increase following the high dose, while hydrolysis to phthalic acid decreased. Repeated dosing did not result in accumulation of DINP and/or its metabolites in blood and tissue but did result in increased formation and elimination of the monoester oxidation products.

In two separate human studies, DINP is rapidly distributed and eliminated in humans, with 30–44% of administered dose recovered over 48 h irrespective of the dose level administered. Unlike rodent metabolism, the human metabolism showed higher rates of DINP ω-1 and ω-2 oxidized metabolites of DINP in urine compared to rats, where the recovered were mainly the hydrolyzed MINP and phthalic acid.

Recent studies conclude that MCIOP is the most abundant metabolite identified in rats and humans and could potentially be used as a biomarker for phthalate metabolism. The metabolite disposition of DINP in the fetus revealed that MINP and its oxidative metabolites along with its glucuronidated form were all present in the fetal plasma, testes, and amniotic fluid. MINP-Gluc was present in higher concentrations in the fetal plasma than the maternal plasma (contradicting observations with other metabolites), indicating potential placental transfer of this MINP-Gluc or—more likely—that conjugation could occur in the fetus by fetal phase II detoxification enzyme systems.

In contrast to absorption following oral exposure, dermal absorption of DINP in adult male F344 rats is low, ranging from 2–4% of the applied dose when measured 7 days after application. This finding agrees with data from other *in vivo* and *in vitro* studies that show absorption of phthalates through rat and human skin decreases as the length of the alkyl chain increases. The dermally absorbed fraction is distributed to multiple tissues, including skin, GI tract, muscle, fat, and liver. The recovery of radioactivity in feces and the GI tract suggests excretion of DINP or its metabolites in the bile suggesting that after absorption, DINP undergoes a similar metabolic fate as orally administered DINP.

The available studies indicate that no greater than 4% of DINP is dermally absorbed in rats. Though there is still little data on DINP on the dermal absorption in humans, it is recognized by other external stake holders (e.g., Danish EPA, ECHA, NICNAS) that absorption of phthalates would be lower in human skin than through rat skin. This is observation is exclusively based on data from *in vitro* migration studies conducted with DEHP and other phthalates. Therefore, EPA considers a dermal absorption of 4% for humans to be appropriate. A similar approach and values were selected by NICNAS 2008 and ECHA 2013a in their assessment report on DINP.

## III. Acute Toxicity

DINP, like other long chain phthalate esters, has low acute oral and dermal toxicity ([ REF _Ref99634655 \h * MERGEFORMAT ]). In a review of acute oral toxicity of phthalates, Krauskopf (1973) reported an acute oral LD50 of greater than 10 g/kg for DINP in rats. Hazleton (1980) reported acute inhalation toxicity (LC50) at >4.4 mg/L; however, the data should be considered with reliability 2 ("reliable with restrictions") because information on measurements of test-chamber atmospheric levels were inadequately reported or because the generating and monitoring procedures were not adequately described. DINP is only minimally irritating to eyes and skin and is not a dermal sensitizer (CPSC 2010).

**Table [ SEQ Table * ARABIC ]. Acute Toxicity and Irritant and Sensitization Properties of DINP (CASRN 68515-48-0)**

| Study Type | Species | Result |
|---|---|---|
| Acute oral toxicity | Rat | $LD_{50}$ >10 g/kg |
| Acute dermal toxicity | Rabbit | $LD_{50}$ >3 g/kg |
| Acute inhalation toxicity | Rats | $LC_{50}$ >4.4 mg/L |
| Primary skin irritation | Rabbit | Slight irritation |
| Primary eye irritation | Rabbit | Slight irritation |
| Dermal sensitization | Rabbit | Not a dermal sensitizer |

## IV. Short-term Toxicity

a) 14-Day Study in Cynomolgus Monkeys (Pugh et al. 2000)

Pugh et al. (2000) evaluated the toxicity of DINP (JAYFLEX plasticizer, CASRN

68515-48-0) in cynomolgus monkeys in a study designed to detect hepatic and other effects. Groups of four young adult male monkeys received either 500 mg/kg/day DINP or the dosing vehicle (0.5% methyl cellulose, 10 mL/kg) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg/day), a known peroxisome proliferator in rodents, was used as a positive control. Treatment with DINP had no apparent adverse effects on body weight, food consumption, or relative weights of the liver, kidney, thyroid/ parathyroid, testes/epididymides, or other organs. DINP-exposed monkeys did not exhibit significant changes in urinalysis, hematology, or clinical chemistry parameters when compared to the controls, with the exceptions of a statistically significant increase in neutrophil count and a statistically significant decrease in lymphocyte count. No distinct treatment-related histopathological effects were observed on the liver, kidney, or testes of animals exposed to DINP; no significant effects were measured on parameters associated with peroxisome proliferation—including peroxisomal beta-oxidation (PBOX, an enzymatic marker), replicative DNA synthesis (an indicator of cell proliferation), or gap junctional intercellular communication as measured by in situ dye transfer in fresh liver slices.

Monkeys treated with clofibrate exhibited occasional emesis (two animals), significantly reduced relative thyroid/parathyroid and testes/epididymides weights, significantly reduced serum calcium, and significantly increased serum triglyceride levels. No other treatment-related effects were reported for animals dosed with clofibrate. These results indicate that peroxisome proliferation did not occur in cynomolgus monkeys treated with DINP or clofibrate under the experimental conditions used in this study.

b) 14-Day Oral Exposure of DINP in Kunming Mice Induces Hepatic and Renal Tissue Injury (Ma et al. 2014).

Ma et al. (2014) administered (oral gavage) DINP (CASRN not reported) to Kunming mice daily for 14-days and then assessed hepatic and renal tissue injury.. Animals were divided randomly into 7 exposure groups of 10 mice each and treated for 14 consecutive days as follows: (1) saline (control), (2) 0.2 mg/kg/day DINP, (3) 2 mg/kg/day DINP, (4) 20 mg/kg/day DINP, (5) 200 mg/kg/day DINP, (6) 50 mg/kg/day melatonin, (7) 50 mg/kg/day melatonin administered 3-hours after dosing with 200 mg/kg/day DINP. Mice were sacrificed after 14 days of exposure, and livers and kidneys were excised for histologic examinations. Additionally, renal and hepatic cell suspensions were prepared and used for measurements of

reactive oxygen species (ROS) production, glutathione (GSH) depletion, malondialdehyde (MDH) content, DNA-protein crosslink (DPC) levels, levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), and interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) levels.

In the liver and kidney, histologic findings were limited to the 20 and 200 mg/kg/day DINP, and the melatonin + DINP treatment groups. Representative histological images from the liver and kidney were provided, however, the overall incidence and severity of histologic findings were not reported and no statistical analysis was conducted. In the liver, oedema was the only finding reported for the 20 mg/kg/day group, while in the 200 mg/kg/day group central vein dilation, oedema, congestion, and narrowing sinusoidal with an extremely loose cytoplasm was reported. In the melatonin + DINP group, hepatic cells were described as "oedemic with increased volumes and narrower sinusoids." In the kidney, histological findings were described as a reduction in the tubular space and oedema of epithelial cells in the glomeruli (20 mg/kg/day DINP), a loss of loop points in the glomerular capillaries (200 mg/kg/day DINP), and glomerular cell proliferation and mild renal tubular epithelia cell oedema (melatonin + DINP). Additional statistically significant effects following exposure to DINP included (1) elevated ROS and DPC levels in the liver and kidney (200 mg/kg/day group); (2) decreased GSH content in the liver (≥20 mg/kg/d DINP) and kidney (200 mg/kg/day only); (3) increased MDA content in the liver (200 mg/kg/day only) and kidney (≥20 mg/kg/d DINP); (4) increased 8-OH-dG content in the liver (200 mg/kg/day) and kidney (≥20 mg/kg/day DINP); and (5) increased IL-1 and TNFα levels in the liver and kidney (≥20 mg/kg/day DINP). In the DINP + melatonin group, ROS, DPC, GSH, MDA,8-OH-dG, IL-1, and TNFα levels in both the liver and kidney were comparable to the control group. Based on these results, study authors concluded that oxidative stress may be involved in the hepatic and renal toxicities associated with DINP exposure, and that melatonin exerts a protective effect.

c) <u>21-Day Study in Fischer-344 Rats (BIBRA 1986; TSCATS Doc # 40-8626208, 1986)</u>

DINP was tested for induction of peroxisome proliferation in a short-term oral study of Fischer-344 rats (5/sex/dose) fed nominal concentrations of 0, 0.6, 1.2, and 2.5% (approximately 639, 1,192, 2,195 mg/kg/day in males; and 607, 1,193, 2,289 mg/kg/day in females) in the diet for 21 days. Parameters monitored included food consumption (measured twice weekly), body weight (measured twice weekly), organ weight (limited to

liver, kidney, and testes), clinical signs, clinical chemistry (limited to triglycerides and total cholesterol), and peroxisome induction.

No test substance-related clinical signs were reported. Mean male body weight was significantly reduced 6-12% in the 1,192 mg/kg/d treatment group starting on study day 7, and 10-28% in the 2,195 mg/kg/d group starting on study day 3. Mean female body weight was significantly reduced 9% in the 607 mg/kg/d group on day 14 only, 6-7% in the 1,193 mg/kg/d group on study days 7 and 10, and 9-14% in the 2,289 mg/kg/d group starting on study day 3. For males, food intake was significantly reduced 19-49% and 10-14% in the 2,195 and 1,192 mg/kg/d groups, respectively throughout the course of the study, while food intake was reduced 41% in high dose females from study days 0 to 3, but then recovered to control levels. Significant dose-dependent increases in absolute and relative liver weight were reported for male (36-132% increase in relative weight) and female (31-137% increase in relative weight) rats across all treatment groups. Similarly, mean relative kidney weight increased in males (14.9-23.9% increase in relative weight) and females (6.7-13.5% increase in relative weight) in all treatment groups. Absolute testis weight was unaffected by treatment with DINP; however, relative testis weight was increased 35% in the highest treatment group. Serum triglycerides were significantly reduced 24-48% in males (≥639 mg/kg/day) and increased 24-26% in females (≥1,193 mg/kg/day), and both effects occurred in a dose-dependent manner. Total cholesterol was significntly reduced 9-24% and 14-24% in males (≥639 mg/kg/day) and females (≥607 mg/kg/day), respectively, however, these effects did not occur dose-dependently. Dose dependent increases in cyanide-insensitive palmitoyl-CoA oxidation levels were reported in males (452-1035%) and females (376-1104%) of the mid- and high-dose groups, while lauric acid 11- and 12-hydroxylase activities were also significantly increased 213-970% for males (≥639 mg/kg/day) and 460-800% for females (≥2,289 mg/kg/day). Total liver protein levels were increased dose-dependently in males and females of all dose groups, while microsomal protein levels were significantly increased in low- and mid-dose (but not high dose) females. Very marked (males) and marked (females) increases in peroxisome proliferation in the liver were evident in rats from the highest treatment group, as evident from electron microscopic examinations, however, these effects were not further quantitatively described.

d) Comparative Toxicological Evaluation of Phthalate Diesters and Metabolites in Sprague-Dawley Male Rats (Kwack et al. 2009)

Kwack et al. (2009) gavaged five-week old (purchased at 4-weeks of age and allowed a 1-week acclimatization period) Sprague-Dawley male rats with nine individual phthalate diesters (DINP, di(2-ethylhexyl) phthalate (DEHP), di(n-butyl) phthalate (DBP), di-n-octyl phthalate (DnOP), diethyl phthalate (DEP), butylbenzyl phthalate (BBP), dimethyl phthalate (DMP), di-isodecyl phthalate (DIDP), diundecyl phthalate (DUP)), five phthalate monoesters (mono(2-ethylhexyl) phthalate (MEHP), monobutyl phthalate (MBuP), monobenzyl phthalate (MBeP), monoethyl phthalate (MEP), monomethyl phthalate (MMP)), and phthalic acid (PA) daily for four weeks. Administered doses were 0 (corn oil vehicle), 250 mg/kg/d (for monoesters and PA) and 500 mg/kd/day (for diesters). Five to six rats were included per treatment group. Animals were observed daily for mortality and clinical signs, body weight was recorded on days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 28, and food consumption was measured twice weekly. On day 28, animals were sacrificed and relative organ weight (heart, lung, liver, kidneys, adrenal glands, spleen, thymus, thyroid glands, testes, epididymides) was recorded (absolute organ weight not reported), blood samples were collected for hematology and clinical chemistry, and sperm counts and motility were determined. Results for the DINP and PA (a metabolite of DINP) studies are of most relevance to this technical review, and therefore only results for these two chemicals are summarized below.

Treatment with DINP and PA did not result in any mortality or changes in food consumption, however, both DINP and PA significantly reduced body weight gain starting approximately 2-weeks after the start of test substance administration. Treatment with DINP significantly increased relative liver weight by 45.3%. No other statistically significant changes in relative organ weight were reported for either DINP or PA. However, absolute organ weight was not reported, and it is preferrable to have measures of both absolute and relative organ weight, particularly for reproductive organs. No hematological parameters were significantly altered following treatment with DINP or PA. Compared to the control, several clinical chemistry parameters were significantly affected by treatment with DINP, including glutamate oxaloacetate transaminase (increased 32%), alkaline phosphatase (increased 260%), and triglycerides (increased 52.5%). Other parameters (i.e., glucose, calcium, blood urea nitrogen, total protein, albumin, total bilirubin, glutamate pyruvate

transaminase, total cholesterol, gamma-gluamyl transferase) were not altered by treatment with DINP, and no clinical chemistry parameters were altered by PA. Treatment with DINP resulted in a 25% decrease in total cauda epididymis sperm count, but did not affect the percentage of motile sperm, however, several sperm motion parameters (including straight-line and curvilinear velocity (VCL)) were reduced. Alternatively, treatment with PA did not affect sperm count or percentage of motile sperm, although VCL was significantly reduced compared to controls. Collectively, these results indicate that short-term exposure to 500 mg/kg/day DINP can cause liver toxicity and effect the male reproductive system.

**Table [ SEQ Table * ARABIC ]. Short-Term Studies in Animals**

| Strain, Dose(s), Route, Duration, Reference | NOAEL (mg/kg bw/d) | LOAEL (mg/kg bw/d) |
|---|---|---|
| Fischer-344 rats (males); 0 or 2.0%; est. 0 or 1,700 mg/kg/day; Diet; 7 days (Bio/dynamics 1982c)[a]<br>DINP-1 (CASRN 68515-48-0) | –[a] | 1,700[a]<br>(Increased absolute and relative liver and kidney weights; macroscopic liver observations (slight congestion); changes in clinical chemistry (i.e., 65% decrease in cholesterol, 90% decrease in triglycerides)) |
| Fischer-344 rats (females); 0, 25, 75, 150, 1,500 mg/kg/day; Gavage; 14 days<br>(Huls AG 1992)[a]<br>DINP-1 (CASRN 68515-48-0)<br>DINP-2 (CASRN 28553-12-0)<br>DINP-3 (CASRN 28553-12-0) | –[a] | 25–75 (LOEL)[a]<br>(Increased dodecanoic acid 12-hydroxylase activity at 25 mg/kg/day for DINP1; 75 mg/kg/day for DINP2 and DINP3) |
| Fischer-344 rats (males); 0, 1,000, 12,000 ppm (0, 1000, and 12,000 mg/kg); Diet; 2 or 4 weeks<br>( et al. 2000)[a]<br>DINP-1 (CASRN 68515-48-0)<br>DINP-A (CASRN 71549-78-5) | 1,000mg/kg (NOEL)[a] | 12,000 mg/kg (LOEL)[a]<br>(Increased relative liver weight (DINP-1, DINP-A); increased peroxisomal beta-oxidation and inhibition of gap junction intracellular communication (DINP-1 and DINP-A at 2 weeks and DINP-1 at 4 weeks); periportal DNA synthesis and centrilobular DNA synthesis increased at 2 weeks (DINP-1 and DINP-A) and at 4 weeks (DINP-A) |
| Fischer-344 rats; 0, 0.6, 1.2, 2.5%; est. 0, 639, 1192, 2,195 mg/kg/day (males); 0, 607, 1,198, 2,289 mg/kg/day (females); Diet; 3 weeks<br>(Barber et al. 1987: BIBRA 1986) | – | 607–639<br>(Increased absolute (24-36%) and relative (31-36%) liver weights (both sexes) compared to controls; increased 11- and 12-hydroxylase activity, hypolipidemic effects, cytoplasmic basophilia in hepatocytes at 1.2%, and eosinophilia at the highest dose) (males/females) |

| Table [ SEQ Table * ARABIC ]. Short-Term Studies in Animals | | |
|---|---|---|
| **Strain, Dose(s), Route, Duration, Reference** | **NOAEL (mg/kg bw/d)** | **LOAEL (mg/kg bw/d)** |
| DINP-1 (CASRN 68515-48-0) | | |
| Fischer-344 rats; 0, 0.2, 0.67, 2%; est. 0, 150, 500, 1,500 mg/kg/day (males); 0, 125, 420, 1,300 mg/kg/day (females); Diet; 28 days<br>(Shellenberg et al. 1983)[a]<br>DINP-2 (CASRN 28553-12-0) | –[a] | 125–150 (LOEL)[a]<br>(Increase in hepatic catalase and carnitine acetyltransferase activity) (males/females) |
| Sprague-Dawley rats (males, 5 weeks old); 0 or 500 mg/kg/day; Gavage; 28 days<br>(Kwack et al. 2009)<br>DINP-2 (CASRN 28553-12-0) | – | 500<br>(Decreased body weight gain; increased (45.3%) relative liver weight; clinical chemistry (i.e., increased glutamate oxaloacetate transaminase (32%), alkaline phosphatase (260%), triglycerides (52.5%)) |
| B6C3F1 mice; 0, 500, 1,500, 4,000, 8,000 ppm; est. 0, 117, 350, 913, 1,860 mg/kg/day (males); 0, 167, 546, 1,272, 2,806 mg/kg/day (females); Diet; 1 or 4 weeks<br>(Kaufmann et al. 2002)[a]<br>DINP-1 (CASRN 68515-48-0) | 117–167<br>(NOEL; males/ females)[a] | 350–546 (LOEL)[a]<br>(Increased absolute (males only) and relative (females only) liver weight; increased peroxisomal volume, and peroxisomal enzyme activity) (males/females) |
| Kunming mice (males, 7-8 weeks old); 0, 0.2, 2, 20, 200 mg/kg/day; Gavage; 14 days<br>(Ma et al. 2014)<br>CASRN not reported | 20 | 200<br>(Liver (central vein dilation, oedema, congestion, narrowing sinusoidal) and kidney (loss of loop points in glomerular capillaries) histology) |
| B6C3F1 mice (males); 0, 500, 6,000 ppm; Diet; 2 or 4 weeks<br>(Smith et al. 2000)[a]<br>DINP-1 (CASRN 68515-48-0) | 500 ppm<br>(NOEL)[a] | 6,000 ppm (LOEL)[a]<br>(Increased relative liver weight and peroxisomal beta-oxidation; hepatic GJIC inhibition) |

| Table [ SEQ Table * ARABIC ]. Short-Term Studies in Animals | | |
|---|---|---|
| **Strain, Dose(s), Route, Duration, Reference** | **NOAEL (mg/kg bw/d)** | **LOAEL (mg/kg bw/d)** |
| B6C3F1 mice; 0, 3,000, 6,000, 12,500, 25,000 ppm; est. 0, 635, 1,377, 2,689, 6,518 mg/kg/day (males); 0, 780, 1761, 3,287, 6,920 mg/kg/day (females); Diet; 4 weeks<br>(Hazleton 1991b)[a]<br>DINP-2 (CASRN 28553-12-0) | –[a] | 635–780[a]<br>(Enlarged and discolored livers; increased incidence of hepatocytomegaly) (males/females) |
| Macaca fascicularis monkeys (males); 0, 500 mg/kg/day; Gavage; 14 days (Pugh et al. 2000)<br>DINP-1 (CASRN 68515-48-0) | 500<br>(NOEL) | No statistically or biologically significant effects were observed after 14 days of exposure at 500 mg/kg/day |
| [a] Studies have been reviewed and summarized previously by various regulatory agencies (see EU RAR 2003; NICNAS 2008; Health Canada 2015a). Studies were not independently reviewed by EPA as part of this technical report. Listed NOAELs/LOAELs were identified in the EU RAR (2003) or in Health Canada (2015a). | | |

## V. Subchronic Toxicity

### a) 13-Week Study in Marmosets (Hall et al. 1999)

Hall et al. (1999) assessed the hepatic peroxisome proliferation potential of DINP (CASRN not reported) in a 13-week study conducted in male and female marmosets. Groups of marmosets (4/sex/dose) received gavage doses of 0, 100, 500, or 2,500 mg/kg/day in a 1% methylcellulose and 0.5% Tween vehicle. A fifth group (3/sex/dose) received 500 mg/kg/day of clofibrate as a positive control for induction of peroxisome proliferation in the liver. Data were collected for clinical signs, food consumption, body weight, ophthalmoscopic examinations, hematology, clinical chemistry, urinalysis, serum testosterone and estradiol, organ weights and histopathology. Cyanide-insensitive palmitoyl CoA oxidase activity, cytochrome P450 concentration, and lauric acid 11- and 12-hydroxylase activities were assayed in hepatic microsomes at the end of the treatment period as markers for peroxisome proliferation.

Oral administration of DINP did not produce any significant evidence of toxicity at doses of 500 mg/kg/day or lower. Treatment-related clinical signs and body weight loss or

suppression of body weight gain were observed in males and females given the 2,500 mg/kg/day dose. There were no clear DINP treatment-related ophthalmoscopic, hematological, serum chemistry, urinalysis, organ weight, or histopathological changes observed in this study. Treatment with DINP appeared to increase relative liver weight, particularly in males (mean ± SD for 0, 100, 500, 2500 mg/kg/d groups: 4.19 ± 0.95, 6.14 ± 2.38, 4.90 ± 1.39, 5.01 ± 1.65, respectively); however, the effect was not dose-dependent and did not reach statistical significance due to variability and small sample size. No statistically significant changes were seen in any marker for peroxisome proliferation when compared with the control values. Treatment with the positive control, clofibrate, increased cyanide-insensitive palmitoyl CoA oxidase activity by approximately 100% ($p < 0.01$) in males and females and increased lauric acid 11-hydroxylase activity in males by 114% ($p < 0.05$). No other changes were statistically significant. These data suggest that DINP did not act as a peroxisome proliferator when administered to marmosets under the conditions of this study.

b) Conclusions on Short-term and Subchronic Toxicity

[ REF _Ref99634749 \h * MERGEFORMAT ] and [ REF _Ref99720235 \h * MERGEFORMAT ] summarize the available short-term and subchronic duration studies of DINP in various species, including rats, mice, dogs, and monkeys. The lowest NOAEL for short-term oral exposure identified for DINP was 20 mg/kg bw/day, based on histopathological changes identified at 200 mg/kg/day in the liver and kidney of mice exposed for 14 days (Ma et al. 2014). The lowest NOAEL for subchronic exposures to rats was 77 mg/kg/day and the LOAEL was 220 mg/kg/day based on increases in kidney and liver weights accompanied by histopathological changes in the liver at the two highest doses and in kidneys (Bio/dynamics 1982a). The lowest NOAEL identified for subchronic oral exposure to dogs was 37 mg/kg/day and the LOAEL was 160 mg/kg/day based on increased absolute and relative liver weights accompanied by histopathological changes at the highest dose tested in males (Hazleton Laboratories 1971b). The lowest NOAEL identified for Marmosets following 13-weeks of oral DINP exposure was 500 mg/kg/day based on a decrease in body weight and body weight gain at the LOAEL of 2,500 mg/kg/day (Pugh et al. 2000 and Huntingdon Life Sciences 1998). No systemic effects were noted in rats

exposed to DINP in one dermal study of 6-week duration (Hazleton 1969).

| Table [ SEQ Table * ARABIC ]. Subchronic Toxicology Studies | | |
|---|---|---|
| **Strain, Dose(s), Route, Duration, Reference** | **NOAEL (mg/kg bw/d)** | **LOAEL (mg/kg bw/d)** |
| Rats; 0, 50, 150, 500 mg/kg/day; Diet; 3 months<br>(Hazleton Laboratories 1971a)[a]<br>DINP-2 (CASRN 28553-12-0) | 150[a]<br>(NOEL; males/ females) | 500[a]<br>(LOEL; Increased absolute (9.3-17.6%) and relative (14.4-25.5%) kidney weight; increased absolute (25.5-29.5%) and relative (29.5-36.2%) liver weight; hepatocytic hypertrophy) (males/females) |
| Fischer-344 rats; 0, 0.1, 0.3, 0.6, 1.0, 2.0%; est. 0, 77, 227, 460, 767, 1,554 mg/kg/day; Diet; 13 weeks<br>(Bio/dynamics 1982a)[a]<br>DINP-1 (CASRN 68515-48-0) | 77[a]<br>(males/ females) | 227[a]<br>(Increased absolute (9.7%) and relative (21.9%) kidney weight; increased relative (% change not reported) liver weight in males; decreased cholesterol level) (males/females) |
| Fischer-344 rats; 0, 2,500, 5,000, 10,000, 20,000 ppm; est. 0, 176, 354, 719, 1545 mg/kg/day (males); 0, 218, 438, 823, 1,687 mg/kg/day; Diet; 13 weeks<br>(Hazleton 1991a)[a]<br>DINP-2 (CASRN 28553-12-0) | –[a] | 176–218[a]<br>(Increase in absolute and relative liver weight accompanied by hepatocellular enlargement in the highest treatment group; increase in absolute and relative kidney weight accompanied by an increase in granular casts and regenerative/basophilic tubules at ≥354-438 mg/kg/day; both sexes) |
| SD rats; 0, 0.3, 1.0%; est. 0, 201, 690 mg/kg/day (males); 0, 251, 880 mg/kg/day (females); Diet; 13 weeks<br>(Bio/dynamics 1982b)[a]<br>DINP-1 (CASRN 68515-48-0) | –[a] | 201–251[a]<br>(LOEL; increase in relative liver weight and triglycerides (both sexes; changes accompanied by a 49-53% increase in alkaline phosphatase (both sexes) and 31% increase in alanine aminotransferase (males only) at the highest dose); increase in absolute and relative kidney weight (both sexes); altered urine chemistry (males only) |
| Wistar rats; 0, 3,000, 10,000, 30,000 ppm: est. 0, 333, 1,101, 3,074 mg/kg/day at day 7; 0, 152, 512, 1,543 mg/kg/day at day 91 (males); 0, 379, 1,214, 3,224 mg/kg/day at day 7; 0, 200, 666, 2,049 mg/kg/day at day 91 (females); Diet; 13 weeks<br>(BASF AG 1987)[a]<br>DINP-2 (CASRN 28553-12-0) | –[a] | 152–200[a]<br>(Clinical chemistry and liver changes related to hepatotoxicity (trend toward reduced triglyceride level and decreased peripheral fat deposits in hepatocytes) (males/females) |

| Table [ SEQ Table * ARABIC ]. Subchronic Toxicology Studies | | |
|---|---|---|
| **Strain, Dose(s), Route, Duration, Reference** | **NOAEL (mg/kg bw/d)** | **LOAEL (mg/kg bw/d)** |
| Sprague-Dawley rats; 0, 1,000, 3,000, 10,000 ppm; est. 0, 60, 180, 600 mg/kg/day; Diet; 13 weeks<br>(Hazleton 1981)[a]<br>DINP-2 (CASRN 28553-12-0) | Not established–[a] | 60[a]<br>(Increased incidence of histopathological lesions in the kidney (i.e., focal mononuclear cell infiltration and mineralization); males only) |
| B6C3F1 mice; 0, 1,500, 4,000, 10,000, 20,000 ppm; est. 0, 365, 972, 2,600, 5,770 mg/kg/day; Diet; 13 weeks<br>(Hazleton 1992)[a]<br>DINP-2 (CASRN 28553-12-0) | 365[a]<br>(males/ females) | 972[a]<br>(Increase in absolute and relative liver weights accompanied with hepatocellular enlargement (≥2,600 mg/kg/day) and other histology at 5,770 mg/kg/day (i.e., pigments in Kupffer cells and bile canaliculi, liver degeneration/necrosis) in both sexes) |
| Marmoset monkeys; 0, 100, 500, 2,500 mg/kg/day; Gavage; 13 weeks<br>(Huntingdon Life Sciences 1998; Hall et al. 1999)<br>DINP-2 (CASRN 28553-12-0) | 500<br>(males/ females) | 2,500<br>(Decrease in body weight and body weight gain; males/females) |
| Dog; 0, 0.125, 0.5, 2.0%: est. 0, 37, 160, 2,000 mg/kg/day; Diet; 13 weeks<br>(Hazleton Laboratories 1971b)[a]<br>DINP-2 (CASRN 28553-12-0) | 37[a]<br>(males) | 160[a]<br>(Increased absolute and relative liver weight (males) accompanied by histopathological changes (i.e., hepatocytic hypertrophy with decreased prominence of hepatic sinusoids) at the highest dose tested in both sexes and increased ALT activity in both sexes) |
| New Zealand White rabbit; 0, 0.5, 2.5 ml/kg: est. 0, 500, 2,500 mg/kg/day; Dermal; 6 weeks<br>(24h application to intact and abraded skin, 5 times per week<br>(Hazleton 1969)[a]<br>DINP-1 (CASRN 68515-48-0) | 2,500[a]<br>(males/females) | No systemic effects were observed at the highest tested dose |
| [a] Studies have been reviewed and summarized previously by various regulatory agencies (see EU RAR 2003; NICNAS 2008; Health Canada 2015a). Studies were not independently reviewed by EPA as part of this technical report. Listed NOAELs/LOAELs were identified in the EU RAR (2003) and in Health Canada (2015a). | | |

## VI. Chronic Toxicity

Chronic toxicity data for DINP (CASRN 68515-48-0) are available from two oral

exposure studies conducted in rats (Lington et al. 1997, Moore 1998a) and one oral exposure study conducted in mice (Moore 1998b). No chronic exposure data on DINP are available for humans or primates.

a) Two-Year Bioassay in Rats (Lington et al. 1997)

Lington et al. (1997) administered DINP (JAYFLEX plasticizer; CASRN 68515-48-0) to Fischer-344 rats (110/sex/group) at dietary levels of 0, 300, 3,000, or 6,000 ppm (corresponding to mean daily intakes of 0, 15, 152, or 307 mg/kg/day in males and 0, 18, 184, or 375 mg/kg/day in females, respectively) for periods up to 24 months. Interim sacrifices of 10 rats/sex/dose were conducted at 6, 12, and 18 months. Surviving animals were sacrificed at 24 months. The rats were observed daily for viability. Clinical observations, body weights, and food consumption were recorded weekly. Hematological, clinical chemistry, and urinalysis parameters were evaluated at 6-, 12-, and 18-month intervals and in 20 rats/sex/concentration at study termination. A complete gross necropsy was conducted on all animals sacrificed at 6, 12, and 18 months and on all survivors at study termination. Tissues, masses, and gross lesions were preserved from animals that died spontaneously or were sacrificed in moribund condition. The adrenals, brain, heart, kidney, liver, ovaries, spleen, testes, and thyroids/parathyroids were weighed prior to fixation at the interim and terminal sacrifices. Gross lesions, tissue masses, and selected tissues, including the liver, kidney, reproductive organs, and thyroid, were examined by light microscopy at the interim sacrifices. At study termination, tissues from all major organs in the high-dose and control groups, plus gross lesions and target tissues (liver and kidney) from the low- and mid-dose groups, were examined by light microscopy. Liver tissues from two rats/sex/group were examined by electron microscopy for morphological evidence of peroxisome proliferation. No measurements of biochemical markers for peroxisome proliferation (e.g., cyanide-insensitive palmitoyl CoA or microsomal lauric acid hydroxylase activities) were performed in this study.

Survival was greater than 60% in all groups at study termination. Survival in the control groups was 75 and 80% for males and females, respectively. Survival in dosed groups ranged from 61–70%. Survival was significantly reduced in mid- and high-dose females when the data were analyzed with the Cox and Kruskall-Wallis tests, but not the Weibull

technique. The most common causes of unscheduled deaths were reported to be mononuclear cell leukemia (MNCL, a neoplastic lesion of the hematopoietic system), and to a lesser extent pituitary neoplasia. Several treatment-related biological effects were noted in mid- or high-dose male and female rats. Effects on the liver and kidney are discussed in detail below. With respect to other treatment-related responses, mean body weight in high-dose males was significantly reduced (4-7%) when compared with controls after 12, 18, and 24 months of treatment. No significant decreases were observed in the mean body weight of females.

Absolute and relative spleen weights were significantly increased at study termination in mid- and high-dose males and high-dose females. Absolute and relative adrenal weights were significantly increased in high-dose females at 6 and 12 months and relative adrenal weights were significantly increased in high-dose males and females at study termination. Possible treatment-related effects on some hematological parameters were reported to occur at study termination. Red blood cell count and mean values for hematocrit and hemoglobin concentration were lower in mid- and high-dose males and females when compared to control values; however, these effects only reached statistical significance in high-dose males. High-dose males and females were also reported to have an increased frequency of nucleated red blood cells, polychromatophilic red cells and reticulocytes. Collectively, these data suggest that DINP treatment induced anemia in high-dose animals. Biologically significant changes in serum chemistry were noted only for indicators of liver function in males; these effects are discussed below. No grossly observable treatment-related effects were evident at the interim sacrifices. At study termination, mid- and high-dose males and high-dose females exhibited an increased incidence of splenic enlargement. Exposure to DINP did not increase the incidence of non-neoplastic histopathological lesions in organs other than the liver.

1. Liver Effects

Male and female rats in the mid- and high-dose groups had statistically significant increases in absolute and relative liver weights throughout most of the treatment period when compared to the controls. There were dose-related increases (1.5- to 3-fold) in serum alkaline phosphatase (ALP), aspartate aminotransaminase (AST), and alanine aminotransaminase (ALT) activities observed in mid- and high-dose males at most time intervals in the study. These liver function indicators did not differ significantly from control values in female rats.

Incidence data for non-neoplastic hepatic lesions showing positive dose-related trends are shown in [ REF _Ref99634892 \h * MERGEFORMAT ]. No treatment-related non-neoplastic lesions were reported in the livers of rats sacrificed after 6 or 12 months of treatment. At 18 months, minimal to slight centrilobular to midzonal hepatocellular enlargement was noted in 9/10 male and 10/10 high-dose males and females, respectively; this effect was absent in the control. Dose-related histopathological changes observed in the liver of male rats at study termination included increases in focal necrosis, spongiosis hepatis, sinusoid ectasia,[1] hepatocellular enlargement, and hepatopathy associated with leukemia ([ REF _Ref99634892 \h * MERGEFORMAT ]). The study authors did not report statistical significance for any of the observed lesions. An independent statistical analysis by Syracuse Research Corporation indicated that the incidence of spongiosis hepatis was significantly increased in mid- and high-dose male rats. The incidences of focal necrosis and hepatocellular enlargement were significantly increased in high-dose males. Statistically significant, dose-related increases were also noted in the incidences of sinusoid ectasia and hepatopathy associated with leukemia in high-dose males. In females, dose-related increases were noted at study termination for focal necrosis, hepatopathy associated with leukemia, and hepatocellular enlargement. The increases in hepatocellular enlargement and hepatopathy associated with leukemia were statistically significant in high-dose females, as determined by the independent analysis. No morphological signs of peroxisome induction were evident in tissue samples examined by electron microscopy.

The NOAEL and LOAEL for hepatic effects in this study were 15 and 152 mg/kg/day, respectively; both are based on a statistically significant increase in the incidence of spongiosis hepatis in mid-dose male rats that was accompanied by increased absolute and relative liver weights and changes in serum enzyme activities.

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[1] The term sinusoid ectasia is a synonym for sinusoidal peliosis hepatis. Sinusoidal peliosis hepatis is frequently associated with the evolution of endothelial liver neoplasms in rodents and humans (Bannasch et al. 2002). The significantly increased incidence of this lesion observed at the highest dose levels of DINP in the male rats studied by Lington et al. (1997) could indicate an early preneoplastic or neoplastic response of the endothelial cells (Bannasch, personal communication). Notably, the original study author may not have considered this relationship when classifying of sinusoid ectasia as a non-neoplastic lesion.

| Table [ SEQ Table * ARABIC ]. Incidence of Selected Non-neoplastic Hepatic Lesions in Fischer-344 Rats Exposed to DINP for 24 Months (Lington et al. 1997) | | | | |
|---|---|---|---|---|
| **Lesion** | **Dose Group mg/kg/day (ppm)** | | | |
| | **Control** | **15 M/18 F (300)** | **152 M/184 F (3,000)** | **307 M/375 F (6,000)** |
| Males (a) | | | | |
| Focal necrosis | 10/81 (12.3%) | 9/80 (11.2%) | 16/80 (20.0%) | 26/80*(32.5%) |
| Spongiosis hepatis | 24/81 (29.6%) | 24/80(30%) | 51/80*(63.8%) | 62/80*(77.5%) |
| Sinusoid ectasia | 16/81 (19.8%) | 16/80 (20.0%) | 24/80 (30.0%) | 33/80*(41.3%) |
| Hepatopathy associated with leukemia | 22/81 (27.2%) | 17/80 (21.3%) | 34/80* (42.5%) | 33/80* (41.3%) |
| Hepatocellular enlargement | 1/81 (1.2%) | 1/80 (1.3%) | 1/80 (1.3%) | 9/80* (11.3%) |
| Females (a) | | | | |
| Focal necrosis | 13/81 (16.0%) | 11/81 (13.6%) | 19/80 (23.8%) | 21/80 (26.3%) |
| Spongiosis hepatis | 4/81 (4.9%) | 1/81 (1.2%) | 3/80 (3.8%) | 4/80 (5.0%) |
| Sinusoid ectasia | 9/81 (11.1%) | 4/81 (4.9%) | 6/80 (7.5%) | 10/80 (12.5%) |
| Hepatopathy associated with leukemia | 16/81 (19.8%) | 18/81 (22.2%) | 24/80 (30.0%) | 33/80* (41.3%) |
| Hepatocellular enlargement | 1/81 (1.2%) | 0/81 (0%) | 0/80 (0%) | 11/80* (13.8%) |

Source: Table 7 in Lington et al. (1997)
Abbreviations: M = male; F = female
* = statistically significant at p#0.05 when compared to the control incidence using Fischer's Exact test;statistical analysis performed by Syracuse Research Corporation.
(a) Number of animals with lesion/total number of animals examined. Percent lesion incidence in parentheses.

2. Kidney Effects

Relative and absolute kidney weights were significantly increased in males and females in the mid- and high-dose groups at most time points. No treatment-related changes in urinalysis parameters or the incidence of non-neoplastic lesions were reported for female rats exposed to DINP. High-dose male rats had significantly increased urine volume relative to controls at all time intervals.

Urinary potassium and glucose were reported to be significantly increased at 6, 12, and 18 (but not 24) months in mid- or high-dose males. Excretion of renal epithelial cells was increased in high-dose males at 6 months, but not following longer treatment periods. Non-neoplastic histological changes in the kidney included a minimal increase in tubular cell pigment in the tubular epithelium of high- dose male rats at the 18-month interim sacrifice. At 24 months, tubular cell pigmentation was increased in severity in animals with advanced MNCL. Chronic progressive nephropathy was reported in most of the study animals; however, the severity of this lesion was not increased by treatment with DINP.

Alpha-2u-globulin accumulation and cell proliferation in the kidney were not assessed in the original study reported by Lington et al. (1997). A retrospective evaluation of archived kidney tissue was conducted to determine whether accumulation of alpha-2u-globulin and cell proliferation occurred in male rats exposed to DINP (Caldwell et al. 1999). Tissue sections obtained from male (all treatment groups) and female (control and high-dose only) rats at the 12-month interim sacrifice were assayed for the presence of alpha-2u-globulin and proliferating cell nuclear antigen (PCNA) using immunohistochemical techniques and computerized image analysis. A dose-dependent increase of alpha-2u-globulin was identified only in the kidney sections from male rats. Although overall cell proliferation did not appear to be increased in male rats, foci of excessive proliferating cells were frequently observed (no incidence data provided) in the renal cortex of high-dose males. The foci of proliferating cells and excessive accumulation of alpha-2u-globulin occurred primarily in the P1 segment of the proximal tubule. Histopathological examination of the archived tissue sections identified tubular regeneration in 6/9, 10/10, 9/10, and 10/10 male rats in the control, low-, mid-, and high-dose groups, respectively. Tubular epithelial hyperplasia was observed in kidney sections from 19 of 20 mid- and high-dose male rats, but not in sections from control or low dose males or control or high-dose female rats. These findings are consistent with the alpha-2u-globulin mechanism of male rat specific nephropathy, which EPA does not regard as relevant to humans.

3. Carcinogenicity

Incidence data for selected preneoplastic and neoplastic lesions are summarized in [

REF _Ref99635267 \h * MERGEFORMAT ]. Treatment with DINP did not result in statistically significant increases in the incidence of any preneoplastic or neoplastic lesion, except MNCL.

Hepatocellular cancer was detected in 3/80 high-dose males, but not at lower doses or in the control group. The incidence of this tumor type was not elevated in dosed female animals when compared to the controls. The combined incidence of neoplastic nodules or hepatocellular cancer was not elevated in either males or females. Renal tubular cell carcinomas were observed in one male in the low-dose group and two males in the high-dose group. Renal transitional cell carcinoma was observed in three male rats in the mid-dose group. No preneoplastic renal lesions were detected in rats of either sex and no neoplastic lesions were detected in the kidneys of female rats.

The incidence of MNCL was statistically significantly increased in the mid- and high-dose groups for both sexes when compared with the concurrent control groups ([ REF _Ref99635267 \h * MERGEFORMAT ]). The incidences of MNCL in the control, low-, mid- and high-dose groups were 41, 35, 60, and 64% for males, and 27, 25, 38, and 54% for females, respectively. As reported by the study authors, MNCL has a significant increasing trend over time and was the most common cause of unscheduled deaths and/or morbidity. In many of the treated rats, MNCL was detected at a very early stage and was limited to an increase in the mononuclear cells in the hepatic sinusoids.

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Preneoplastic and Neoplastic Lesions in Fischer-344 Rats Exposed to DINP for 24 Months (Lington et al. 1997)**

| Lesion | Dose Group mg/kg/day (ppm) | | | |
|---|---|---|---|---|
| | **Control** | **15 M/18 F (300)** | **152 M/184 F (3,000)** | **307 M/375 F (6,000)** |
| Males (a) | | | | |
| Liver: neoplastic nodules | 3/81 (3.7%) | 1/80 (1.3%) | 1/80 (1.3%) | 1/80 (1.3%) |
| Liver: hepatocellular cancer | 0/81(0%) | 0/80(0%) | 0/80(0%) | 3/80 (3.8%) |
| Liver: neoplastic nodules or cancer Combined | 3/81 (3.7%) | 1/80 (1.3%) | 1/80 (1.3%) | 4/80 (5.0%) |
| Kidney: transitional cell carcinoma | 0/81(0%) | 0/80(0%) | 3/80 (3.8%) | 0/80(0%) |

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Preneoplastic and Neoplastic Lesions in Fischer-344 Rats Exposed to DINP for 24 Months (Lington et al. 1997)**

| Lesion | Dose Group mg/kg/day (ppm) | | | |
|---|---|---|---|---|
| | Control | 15 M/18 F (300) | 152 M/184 F (3,000) | 307 M/375 F (6,000) |
| Kidney: transitional cell adenoma | 0/81(0%) | 0/80(0%) | 0/80(0%) | 0/80(0%) |
| Kidney: tubular cell carcinoma | 0/81(0%) | 1/80 (1.3%) | 0/80(0%) | 2/80 (2.5%) |
| Kidney: tubular cell adenoma | 0/81(0%) | 0/80(0%) | 0/80(0%) | 0/80(0%) |
| Hematopoietic System: mononuclear cell leukemia(MNCL) | 33/81 (41%) | 28/80 (35%) | 48/80* (60%) | 51/80* (64%) |
| Females (a) | | | | |
| Liver: neoplastic nodules | 0/81(0%) | 2/81 (2.5%) | 0/80(0%) | 1/80 (1.3%) |
| Liver: hepatocellular cancer | 1/81 (1.2%) | 0/81(0%) | 0/80(0%) | 1/80 (1.3%) |
| Liver neoplastic nodules or carcinoma combined | 1/81 (1.2%) | 2/81 (2.5%) | 0/80 (0%) | 2/80 (2.5%) |
| Kidney: transitional cell carcinoma | 0/81(0%) | 0/81(0%) | 0/80(0%) | 0/80(0%) |
| Kidney: transitional cell adenoma | 0/81(0%) | 0/81(0%) | 0/80(0%) | 0/80(0%) |
| Kidney: tubular cell carcinoma | 0/81(0%) | 0/81(0%) | 0/80(0%) | 0/80(0%) |
| Kidney: tubular cell adenoma | 0/81(0%) | 0/81(0%) | 0/80(0%) | 0/80(0%) |
| Hematopoietic System: mononuclear cell leukemia (MNCL) | 22/81 (27%) | 20/81 (25%) | 30/80* (38%) | 43/80* (54%) |

Source: Table 8 in Lington et al. (1997)
Abbreviations: M = male; F = female
* = statistically significant at p#0.05 when compared to the control incidence using Fisher's Exact test; statistical analysis performed by Lington et al. (1997).
(a) number of animals with lesion/ total number of animals examined. Percent lesion incidence in parentheses.

b) Two-Year Bioassay in Rats (Moore 1998a)

Moore (1998a, TSCATS Doc # 89980000308) investigated the chronic oral toxicity and carcinogenicity of DINP in an EPA guideline- and GLP-compliant study conducted in Fischer-344 rats (5–55/sex/concentration/time point) using the study design

outlined in [ REF _Ref99635387 \h * MERGEFORMAT ] below. DINP (CASRN 068515-48-0) was administered at dietary concentrations of 0, 500, 1,500, 6,000 and 12,000 ppm (average daily doses of 0, 29, 88, 359, 733 mg/kg/day in males, and 0, 36, 109, 442, 885 mg/kg/day in females) for 105 weeks. Additional groups of male and female rats were given 12,000 ppm (637 and 774 mg/kg/day, respectively) for 78 weeks and received basal diet only for the remainder of the study (26 weeks) in order to evaluate the reversibility of DINP toxicity. Compound WY 14643 (a widely used activator of peroxisome proliferator activator receptors [PPARs]) was administered as a positive control to 15 male rats for up to 13 weeks.

Hepatocellular proliferation rates and biochemical analyses (protein concentration, cyanide insensitive palmitoyl CoA oxidation, and DNA concentration) were measured in 5 rats/sex selected from Groups 1 to 5 and 7 during study weeks 1, 2, and 13; and on 5 rats/sex from Groups 1, 4, and 5 during study week 104. At week 79, 5 rats/sex/group from Groups 1, 4, and 5 were implanted with osmotic minipumps and evaluated for possible hepatocellular proliferation and biochemical parameters, and 10 rats/sex/group were sacrificed for histological evaluation. The test animals were observed daily for clinical signs. Body weights were recorded weekly for weeks 1 to 17 and once every 4 weeks thereafter. Urinalysis, hematology, and clinical chemistry parameters were evaluated in 10 rats/sex/concentration at 26, 52, 78, and 104 weeks. Necropsy was performed on all animals found dead or sacrificed in extremis and animals sacrificed after at least 78 or 104 weeks of treatment. Organ weights were collected for brain, kidneys, liver, lung, spleen, testes, and uterus. Gross lesions and a comprehensive set of tissues were preserved for histopathological analysis.

**Table [ SEQ Table * ARABIC ]. Group Assignment and Dietary Dose Levels in the Two-Year Bioassay in Fischer-F344 Rats Reported by Moore (1998a)**

| Group No. | Group Designation | Dietary Level (ppm) | Estimated Dose M/F (mg/kg/day) | Week of Sacrifice | | | | | Total RatsM/F |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 M/F (a) | 2 M/F (a) | 13 M/F (a) | 79 M/F (b,c) | 104–106 M/F (a,b) | |
| 1 | Control | 0 | 0/0 | 5/5 | 5/5 | 5/5 | 15/15 | 55/55 | 85/85 |
| 2 | Low | 500 | 29/36 | 5/5 | 5/5 | 5/5 | 0 | 55/55 | 70/70 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 3 | Mid-Low | 1500 | 88/109 | 5/5 | 5/5 | 5/5 | 0 | 55/55 | 70/70 |
| 4 | Mid-High | 6,000 | 359/442 | 5/5 | 5/5 | 5/5 | 15/15 | 55/55 | 85/85 |
| 5 | High | 12,000 | 733/885 | 5/5 | 5/5 | 5/5 | 15/15 | 55/55 | 85/85 |
| 6 | Recovery-High (d) | 12,000 (weeks 1–78) | 637/774 | 0 | 0 | 0 | 0 | 55/55 | 55/55 |
| 7 | Positive Control | 1000 (WY14643) | NR | 5/0 | 5/0 | 5/0 | 0 | 0 | 15/0 |

Source: Modified from an unnumbered table on page 18 of Moore (1998a)
Abbreviations: M = male; F = female
(a) Hepatocellular proliferation rates and biochemical analyses (protein concentration, cyanide insensitive palmitoyl CoA oxidation and DNA concentration) were measured in 5 rats/sex from Groups 1–5 and 7 during study weeks 1, 2, and 13 and on 5 rats/sex from Groups 1, 4, and 5 during study week 104.
(b) Five rats/sex/group were implanted with osmotic minipumps and evaluated for possible hepatocellular proliferation andbiochemical parameters. The remaining rats within each group were processed for histological evaluation.
(c) Ten rats/sex/group designated for histological evaluation; the remaining rats were evaluated for possiblehepatocellular proliferation and biochemical parameters
(d) Rats in Group 6 were removed from the test diet on study week 78 and placed on basal diet for the remainder of the study.

Survival of males and females was unaffected by treatment through week 78 and was greater than or equal to 93% in all groups. Percent survival in Groups 1 through 6 was 74, 71, 78, 66, 54, and 58% in the males and 76, 80, 80, 71, 66, and 70% in the females, respectively. Survival was statistically significant reduced in the 12,000 ppm males when compared to the same sex controls. Clinical signs that were (in general) proportional to dose included thin appearance, hunched posture, hypoactivity, prostration, pale body appearance, rough hair coat, urine stains, and few and/or no feces (primarily seen in males). Mean body weights of male and female rats in Groups 5 and 6 were generally lower than the controls during most of the study period. The decrements in body weights were (in general) statistically significant from weeks 5 to 105 for males and from weeks 2 to 105 for females. Body weights in the recovery groups rebounded after cessation of treatment.

Significant changes in hematological parameters included mild decreases in the mean values for erythrocyte count, hemoglobin, and hematocrit in Groups 5 and 6 at most intervals. These changes were not accompanied by concurrent significant increases in mean values for reticulocyte percentages or absolute counts. The mean values for hematological parameters in the recovery group (Group 6) were not (in general) significantly different from the control at study termination. At necropsy, enlarged spleen, dark area of the stomach and uterine pathologies (cyst, mass, or thickened wall) were

observed to have a dose-related increase in Groups 4 and 5. Absolute and relative spleen weights were significantly increased in Group 5 at weeks 79 (males and females) and Groups 4 and 5 at week 104 (females only). No statistically significant increases in the incidence of non-neoplastic lesions were reported for organs other than the liver and kidney (results for these organs are discussed in detail below).

1. Liver Effects

Absolute and relative liver weights were significantly increased at the 6,000 and 12,000 dietary concentrations at weeks 1, 2, 13, 79, and 104 in both sexes. Livers that appeared enlarged and/or granular/pitted/rough were observed with the greatest frequency in the 6,000 ppm, 12,000 ppm, and recovery groups (including unscheduled deaths, killed at week 79, and at study termination). Mean liver weights in the 12,000-ppm recovery group were comparable to control values when measured at the end of the 26-week recovery period, suggesting that liver enlargement was reversible. Serum enzyme activities (AST, ALT) were significantly increased in both sexes at the two highest doses at weeks 52, 78, and 104, with no indication of reversibility in the high-dose recovery groups. The changes in enzymatic activity were associated with histological evidence of liver toxicity. The mean values for palmitoyl-CoA oxidase activity, an indicator of peroxisome proliferation, were significantly increased in the livers of 12,000 ppm male and female rats at all time points. Palmitoyl CoA oxidase was also significantly increased in 6,000 ppm female (but not male) rats at 104 weeks—the only time point measured for this dietary concentration. Palmitoyl-CoA oxidase activity was not evaluated in the high-dose recovery group; thus, reversibility of this endpoint could not be assessed. After 1 week of treatment, significant increases were noted for the number of mitotic cells and mean labeling index for hepatocytes from all rats evaluated in the high-dose group (5/sex). The number of mitotic cells and labeling index were not increased in high-dose males or females at 2, 13, or 104 weeks compared to controls, suggesting that sustained cell proliferation was not maintained after the first week. Large increases were observed in the labeling index in positive control animals at week 1, but questionable responses were observed at week 2 and 13.

Incidences of selected non-neoplastic hepatic lesions are summarized in [ REF

_Ref99635452 \h * MERGEFORMAT ]. The most sensitive histopathological response to DINP exposure was increased incidence of spongiosis hepatis, an apparent degenerative lesion of perisinusoidal (Ito) cells. Spongiosis hepatis was significantly increased in male rats at dietary concentrations of 6,000 and 12,000 ppm. A dose-related response was not observed in females. Slight diffuse hepatocellular enlargement was evident in all high-dose rats (5/sex) after week 2 of treatment. Diffuse hepatocellular enlargement (moderate mean severity in males and slight mean severity in females) was evident in all high-dose animals (5/sex) sacrificed after 13 weeks and in 10/10 high-dose males and 9/10 high-dose females after 79 weeks. At study termination, diffuse hepatocellular enlargement was significantly increased in high-dose males (17/55) and females (33/55) when analyzed independently by Syracuse Research Corporation. Other lesions that showed a statistically significant response in the 12,000 ppm dose groups at study termination were cytoplasmic eosinophilia in males and females (first detected at week 13), increased pigment in Kupffer cell canaliculi in males and females (first detected at week 79), and slightly increased individual cell necrosis/degeneration in males at 12,000 ppm.

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Hepatic Lesions in Fischer-344 Rats Exposed to DINP in the Diet for Two Years (Moore 1998a)**

| Lesion | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **29 M/ 36 F (500)** | **88 M/ 109 F (1,500)** | **359 M/ 442 F (6,000)** | **733 M/ 885 F (12,000)** | **Recovery (a) 637 M/ 774 F (12,000)** |
| Males | | | | | | |
| Spongiosis Hepatis | 5/55 (b) (9.1%) | 5/50 (10.0%) | 2/50 (4.0%) | 13/55* (23.6%) | 21/55* (38.2%) | 9/50 (18.0%) |
| Cytoplasmic Eosinophilia | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 0/55 (0%) | 31/55* (56.4%) | 0/50 (0%) |
| Diffuse hepatocellular enlargement | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 0/55 (0%) | 17/55* (30.9%) | 0/50 (0%) |
| Increased Pigment | 1/55 (1.8%) | 0/50 (0%) | 1/50 (2.0%) | 0/55 (0%) | 7/55* (12.7%) | 4/50 (8.0%) |
| Individual cell degeneration/ necrosis | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 1/55 (1.8%) | 5/55* (9.1%) | 0/50 (0%) |
| Females | | | | | | |
| Spongiosis | 0/55 | 0/50 | 0/50 | 1/55 | 2/55 | 0/50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Hepatis | (0%) | (0%) | (0%) | (1.8%) | (3.6%) | (0%) |
| Cytoplasmic Eosinophilia | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 0/55 (0%) | 35/55* (63.6%) | 0/50 (0%) |
| Diffuse hepatocellular enlargement | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 0/55 (0%) | 33/55* (60.0%) | 0/50 (0%) |
| Increased Pigment | 7/55 (12.7%) | 8/50 (16.0%) | 9/50 (18.0%) | 5/55 (9.1%) | 16/55* (29.1%) | 10/50 (20.0%) |
| Individual cell degeneration/ necrosis | 0/55 (0%) | 0/50 (0%) | 0/50 (0%) | 0/55 (0%) | 0/55 (0%) | 0/50 (0%) |

Source: Tables 10A and 10C in Moore (1998a)
Abbreviations: M = male; F = female
* = significantly different from control (p#0.05) by Fisher's Exact test as performed by Syracuse Research Corporation.
(a) The 12,000 ppm recovery group received 12,000 ppm DINP in the diet for 78 weeks, followed by a 26-week recovery period during which the test animals received basal diet alone.
(b) Number of animals with lesion/number of animals with livers examined; percentage is given in parentheses. Incidence is sum of lesions observed in unscheduled deaths and at terminal sacrifice.

2. Kidney Effects

Blood urea nitrogen (BUN), a biomarker for kidney toxicity, was (generally) significantly increased at weeks 26, 52, 78, and 104 in male and female rats exposed to 6,000 (Group 4) or 12,000 ppm (Group 5). The increases were within 32% of the control values in 6000 ppm rats and within 50% for the 12,000 ppm rats. BUN remained elevated in males in the 12,000 ppm recovery group (Group 6) and was comparable to the level observed in Group 5 rats at week 104. In contrast, BUN in Group 6 females was comparable to the same sex control value after 26 weeks of recovery. Absolute and relative kidney weights were significantly increased in the 6,000 and 12,000 ppm males and females at 79 weeks and in 6,000 ppm females (but not males) and 12,000 ppm males and females at 104 weeks. Kidney weights in the 12,000 ppm recovery groups were comparable to the same-sex control values at the end of the 26-week recovery period. At necropsy, a dark appearance of the kidneys was noted in 6,000 and 12,000 ppm males and females. Treatment-related increases in the severity of tubule cell pigment occurred in the kidneys of males at 12,000 ppm ([ REF _Ref99635605 \h * MERGEFORMAT ]). Kidney changes at 104 weeks consisted of a dose-related increase in incidence and severity of mineralization of the renal papilla in males in the 6,000, 12,000 and 12,000 ppm recovery groups and an increased incidence and severity of increased pigment tubule cell at 6,000 and 12,000 ppm in both sexes ([ REF _Ref99635605 \h * MERGEFORMAT ]).

**Table [ SEQ Table * ARABIC ]. Incidence and Severity of Selected Non-neoplastic Lesions in the Kidneys of Male Fischer-344 Rats Fed DINP for Two Years (Moore 1998a)**

| | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **29 M/ 36 F(500)** | **88 M/ 109 F (1500)** | **359 M/ 442 F (6000)** | **733 M/ 885 F (12,000)** | **Recovery (a) 637 M/ 774 F (12,000)** |
| Number examined (b) | 36 | 35 | 39 | 31 | 27 | 29 |
| Mineralization of renal papilla | | | | | | |
| Minimal | 6 | 11 | 9 | 6 | 2 | 0 |
| Slight | 0 | 0 | 0 | 24 | 1 | 2 |
| Moderate | 0 | 0 | 0 | 0 | 22 | 27 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Total | 6 | 11 | 9 | 30 | 25 | 29 |
| Tubule cell pigment | | | | | | |
| Minimal | 24 | 21 | 18 | 0 | 0 | 0 |
| Slight | 10 | 12 | 21 | 23 | 7 | 26 |
| Moderate | 0 | 1 | 0 | 6 | 17 | 3 |
| Moderately severe | 0 | 1 | 0 | 2 | 3 | 0 |
| Total | 34 | 35 | 39 | 31 | 27 | 29 |

Source: Text Table 3 on page 65 of Moore (1998a)
Abbreviation: M = Male, F = Female
(a) The 12,000-ppm recovery group received 12,000 ppm DINP in the diet for 78 weeks, followed by a 26-weekrecovery period during which the test animals received basal diet alone.
(b) Number examined at terminal sacrifice; does not include unscheduled deaths.

3. Carcinogenicity

Moore (1998a) did not report results for statistical analyses of tumor incidence data. An independent analysis of the tumor data has been performed by the Chronic Hazard Advisory Panel (CHAP) for the U.S. Consumer Product Safety Commission (CHAP 2001). The CHAP extracted individual animal data from the study report, which were subsequently analyzed by the National Toxicology Program (NTP) using six statistical procedures (Life Table, Poly 3, Poly 1.5, Poly 6, Logistic Regression, and Cochrane-Armitage/Fisher's Exact) that have been validated in several chronic and cancer bioassays. The selection of results from a particular procedure depended, in part, on whether the lesion of interest was lethal or nonlethal. Results from the CPSC/NTP analysis for selected tumors are shown in [ REF _Ref99635698 \h * MERGEFORMAT ].

The overall incidences (i.e., the sum of lesion incidences in unscheduled deaths, the interim sacrifice if performed, and at terminal sacrifice) of hepatocellular carcinoma and combined carcinoma and adenomas were significantly increased in 12,000 ppm males ([ REF _Ref99635698 \h * MERGEFORMAT ]) with a significant dose-related trend. The incidence of combined carcinoma and adenomas was significantly increased in 12,000 ppm females, also with a dose-related trend. Hepatocellular carcinoma was first observed in high-dose males at the interim sacrifice and in other dose groups at the terminal sacrifice.

Malignant renal tubule cell carcinoma was detected in each of two male rats in the

12,000 ppm (733 mg/kg/day) group and in four males in the recovery group. The increased incidence of malignant tubule cell carcinoma in the recovery group was statistically significant when compared to the controls ([ REF _Ref99635698 \h * MERGEFORMAT ]).

The incidences of MNCL in male and female rats receiving the 6,000 and 12,000 ppm concentrations were significantly increased ([ REF _Ref99635698 \h * MERGEFORMAT ]) with statistically significant dose-related trends. The incidences of MNCL in the recovery groups were also significantly greater than in the controls. There is some evidence that the onset of MNCL was earlier in treated males. MNCL was first detected in the 6,000-ppm group via an unscheduled death at study day 352. In comparison, the MNCL was first detected in the control group at an interim sacrifice at day 549. Decreases in hemoglobin concentration and red blood cell numbers and a statistically significant increase in mean spleen weight in both male and female rats were correlated with the incidence of MNCL. Between 31 and 60% of unscheduled deaths in the study were attributable to MNCL ([ REF _Ref99635794 \h * MERGEFORMAT ]), demonstrating that this lesion is life-threatening in rats treated with DINP.

**Table [ SEQ Table * ARABIC ]. Overall incidence of selected tumors in Fischer-344 rats exposed to DINP in the diet (Moore 1998a) (a)**

| Lesion | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **29 M/ 36 F (500)** | **88 M/ 109 F (1,500)** | **359 M/ 442 F (6,000)** | **733 M/ 885 F (12,000)** | **High Dose/ Recovery (b) 637M/774 F (12,000)** |
| Males | | | | | | |
| Hepatocellular adenoma | 4/65 (c) (6%) | 3/50 (6%) | 2/50 (4%) | 6/65 (9%) | 6/65 (15%) | – (d) |
| Hepatocellular carcinoma | 1/65 (2%) | 0/50 (0%) | 0/50 (0%) | 1/65 (2%) | 12/65* (18%) | – |
| Hepatocellular carcinoma or adenoma (combined) | 5/65 (8%) | 3/50 (6%) | 2/50 (4%) | 7/65 (11%) | 18/65* (28%) | – |
| Renal tubular carcinoma | 0/65 (0%) | 0/55 (0%) | 0/55 (0%) | 0/65 (0%) | 2/65 (3%) | 4/50* (e) (8%) |
| MNCL (hematopoietic system) | 22/65 (34%) | 23/50 (46%) | 21/50 (42%) | 32/65* (49%) | 30/65* (46%) | 31/50* (f) (62%) |
| Females | | | | | | |

**Table [ SEQ Table * ARABIC ]. Overall incidence of selected tumors in Fischer-344 rats exposed to DINP in the diet (Moore 1998a) (a)**

| Lesion | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 29 M/ 36 F (500) | 88 M/ 109 F (1,500) | 359 M/ 442 F (6,000) | 733 M/ 885 F (12,000) | High Dose/ Recovery (b) 637M/774 F (12,000) |
| Hepatocellular adenoma | 0/65 (0%) | 1/49 (2%) | 0/50 (0%) | 1/65 (2%) | 3/65 (5%) | – |
| Hepatocellular carcinoma | 1/65 (2%) | 0/49 (0%) | 0/50 (0%) | 1/65 (2%) | 5/65 (8%) | – |
| Hepatocellular carcinoma or adenoma (combined) | 1/65 (2%) | 1/49 (2%) | 0/50 (0%) | 2/65 (3%) | 8/65* (12%) | – |
| MNCL | 17/65 (26%) | 16/49 (33%) | 9/50 (18%) | 30/65* (46%) | 29/65* (45%) | 24/50 * (f) (48%) |

Source: CHAP (2001) text pages 68–71 and Appendix B Abbreviations: M = male; F = female

* = statistically significant at p#0.05 by one or more of the following: Fisher's Exact test, Poly-3, Logistic Regression, or Life Table analysis.

(a) Analysis of individual animal data as performed by the National Toxicology Program and reported in the textand Appendix B of CHAP (2001)

(b) The high dose/recovery group received 12,000 ppm for 78 weeks, followed by a 26-week recovery periodduring which they received basal diet alone.

(c) Number of animals with neoplasm/ total number of animals examined. Percent tumor incidence in parentheses. Based on extraction and analysis of individual animal data as reported in CHAP (2001).

Overall incidence for control, 6,000 ppm and 12,000 ppm groups (n = 65) includes incidence data for unscheduleddeaths, interim sacrifice at week 78 and terminal sacrifice. Overall incidence for the remaining groups includes incidence data for unscheduled deaths and terminal sacrifice.

(d) Neither NTP nor CHAP (2001) performed statistical analysis of tumor incidence.

(e) Changes in the incidence of this lesion were statistically significant as determined by CHAP (2001).

(f) Statistical significant at p#0.05 by Fisher's Exact test conducted by Syracuse Research Corporation.

**Table [ SEQ Table * ARABIC ]. MNCL as a Cause of Unscheduled Death in F-344 Rats Exposed to DINP in the Diet (Moore 1998a)**

| Sex | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 29 M/ 36 F (500) | 88 M/ 109 F (1,500) | 359 M/ 442 F (6,000) | 733 M/ 885 F (12,000) | Recovery (a) 637M/774 F (12,000) |
| Males | 7/22 (b) (32%) | 8/23 (35%) | 7/21 (33%) | 16/32 (50%) | 18/30 (60%) | 14/31 (45%) |
| Females | 7/17 (41%) | 5/16 (31%) | 3/9 (33%) | 12/29 (41%) | 13/30 (43%) | 12/24 (50%) |

Source: Compiled from incidence data and death comments in Table 10E (pages 365 and 381) in Moore (1998a)

Abbreviations: M = male; F = female

(a) The high dose/recovery group received 12,000 ppm for 78 weeks, followed by a 26-week recovery periodduring which test animals received basal diet alone
(b) Number of deaths attributed to MNCL/ total number of deaths; percentage of deaths attributable to MNCL inparentheses.

c) Two-Year Bioassay in Mice (Moore 1998b)

The chronic toxicity and carcinogenicity of DINP were investigated in a standard guideline and GLP-compliant two-year oral bioassay conducted in B6C3F1 mice (Moore 1998b; TSCATS Doc # 89980000308). The study design for this investigation is summarized below in [ REF _Ref99635897 \h * MERGEFORMAT ]. DINP (CASRN 068515-48-0) was administered in the diet to groups of male and female mice (55/sex/dose) at concentrations of 0, 500, 1,500, 4,000, or 8,000 ppm (designated as Groups 1 through 5, respectively) for at least 104 weeks. These concentrations corresponded to average daily doses of 0, 90, 276, 742, 1,560 mg/kg/day in males and 0, 112, 336, 910, 1,888 mg/kg/day in females. Fifteen mice/sex/group were exposed to the same dietary concentrations and sacrificed at 79 weeks. Additional groups of male and female mice (55/sex; designated as Group 6) were given 8,000 ppm (1,377 and 1,581 mg/kg/day, in males and females, respectively) for 78 weeks and then given basal diet only for 26 weeks (total time on study 104 weeks) to evaluate the reversibility of DINP-induced toxicity. Five mice per sex in Groups 1 through 5 were designated for evaluation of hepatocellular proliferation at 78 weeks and 5 mice/sex in treatment 1–6 were evaluated for evaluation of hepatocellular proliferation after 104 weeks.

The test animals were observed daily for clinical signs and twice daily for mortality and morbidity. Body weights were recorded weekly for weeks 1 to 17 and once every 4 weeks thereafter. Urinalysis, hematology, and clinical chemistry parameters were evaluated in 10 rats/sex/concentration at weeks 26, 52, 78, and 104. Necropsy was performed on all animals found dead or sacrificed in extremis and animals sacrificed after at least 78 or 104 weeks of treatment. Organ weights were collected for brain, kidneys, liver, lung, spleen, testes, and uterus. At week 79, microscopic evaluation was performed on complete sets of tissues from 10 mice/sex/group in the control and 8,000 ppm groups and on liver, testes with epididymides (males), uterus (females), spleen, kidneys, and gross lesions in the 500, 1,500, and 4,000 ppm groups. At study termination, microscopic evaluation was performed on complete sets of tissues from all surviving mice in the

control and 8,000 ppm groups (excluding those designated for evaluation of hepatocellular proliferation) and from all mice that died or were sacrificed in moribund condition during the study. In addition, the liver, testes with epididymides (males), uterus (females), spleen, kidneys and gross lesions were examined in mice in the 500, 1,500, 4,000, and 8,000 ppm recovery groups.

**Table [ SEQ Table * ARABIC ]. Group Assignment and Dietary Dose Levels in the Two-Year Bioassay in B6C3F1 Mice Reported by Moore (1998b)**

| Group No. | Group Designation | Dietary Level(ppm) | Estimated Dose M/F (mg/kg/day) | Week | | Total Mice M/F |
|---|---|---|---|---|---|---|
| | | | | 79 M/F (a,b) | 105–106 M/F (a,b) | |
| 1 | Control | 0 | 0/0 | 15/15 | 55/55 | 70/70 |
| 2 | Low | 500 | 90/112 | 15/15 | 55/55 | 70/70 |
| 3 | Mid-Low | 1.500 | 276/336 | 15/15 | 55/55 | 70/70 |
| 4 | Mid-High | 4.000 | 742/910 | 15/15 | 55/55 | 70/70 |
| 5 | High | 8.000 | 1,560/1,888 | 15/15 | 55/55 | 70/70 |
| 6 | Recovery-High (c) | 8,000 (weeks 1–78) | 1,377/1,581 | 0 | 55/55 | 55/55 |

Source: Modified from an unnumbered table on page 16 of Moore (1998b)
Abbreviations: M, male; F, female

(a) Hepatocellular proliferation rates and biochemical analyses (protein concentration, cyanide insensitive palmitoyl CoA oxidation and DNA concentration) were measured during study weeks 79 and 105–106.

(b) Five mice/sex in Groups 1 and 5 were implanted with osmotic minipumps; the test animals were sacrificed and processed for evaluation of hepatocellular proliferation and biochemical parameters. Five mice/sex in groups 2, 3, 4, and 6 (week 105–106 only for Group 6) were implanted with osmotic minipumps; the test animals were sacrificed and processed for evaluation of hepatocellular proliferation and biochemical parameters (the hepatocellular tissues were processed to the block stage). The remaining mice within each group at week 79 were processed for histomorphological evaluation.

(c) Mice in Group 6 were removed from the test diet on study week 78 and placed on basal diet for the remainder of the study.

The survival rate of 8,000 ppm males was significantly decreased for weeks 1 to 104. At study termination, survival in the 8,000 ppm males was 63% compared to 87% in controls. In females, survival was not affected in a dose-dependent manner; survival was lowest in the 4,000-ppm group (62% compared to 81% in the controls), while survival in 8,000 ppm females was 77%. The most common causes of death in these groups were hepatocellular neoplasia and lymphoma in both sexes and fibrosarcoma and hemangiosarcoma in females. Mean body weights were significantly decreased in males

in the 4,000 and 8,000 ppm groups from Week 29 to study termination. Mean body weights in the recovery group males were decreased at weeks 15 to 105. In females, significant reductions in mean body weight were observed in the 1,500, 4,000, and 8,000 ppm and recovery groups for most of the study. Females in the 500 ppm group had significantly reduced mean body weights at weeks 8, 21, and 25. Body weight gain increased in the recovery groups when treatment with DINP was discontinued. Clinical signs that showed a treatment-related response included hunched posture (highest incidence in 8,000 ppm animals), hypoactivity (highest incidence in 8,000 ppm males and 4,000 ppm females), few feces (highest incidence in 8,000 ppm females), and urine stains (predominately in 8,000 ppm males). Increased incidences of swelling in the ventral-abdominal region were observed in the 4,000 and 8,000 ppm and recovery males as well as in the 8,000 ppm and recovery females. These incidences appeared to correlate with the incidence of animals with liver masses at necropsy. Total mean food consumption values for dosed groups were generally comparable to or higher than the controls.

The findings for organ weights, gross examination, and histopathology were consistent with the liver and kidney as the principal target organs for DINP toxicity. The results for these organs are discussed in detail below. With respect to effects on other tissues and organs, males, and females in the 8,000 ppm and recovery groups had treatment-related decreases in leukocyte, lymphocyte, and/or segmented neutrophil counts. Dose-related effects on organ weights included decreased testis weight (absolute and relative to brain weight) without histologic correlates in the 500, 1500, and 8,000 ppm groups; increased relative lung weight (relative to body weight) in 8,000 ppm and recovery group males; and increased relative brain weight in 4,000 and 8,000 ppm and recovery group males. Gross findings in other organs included lung masses (primarily in male mice) and enlarged spleen (predominately in female mice). Microscopic evaluation of the lung masses generally revealed alveolar/bronchiolar neoplasms that were unrelated to treatment.

Enlargement of the spleen was most frequently related to increased extramedullary hematopoiesis or to involvement by neoplasia. No treatment-related microscopic lesions were identified in organs other than the liver or kidneys.

1. Liver Effects

Gross and microscopic evidence of liver toxicity were observed in dosed animals of both sexes. At interim sacrifice, absolute and relative liver weights were increased in 4,000 ppm males and females (statistical significance achieved for increased relative weight in 4,000 ppm males) and significantly increased in 8,000 ppm males. At study termination, absolute and relative liver weights were significantly increased in 4,000 and 8,000 ppm males. Relative liver weight was significantly increased in the recovery group. Absolute and relative liver weights were increased in females in the 4,000 and 8000 ppm and recovery groups, but the responses were not statistically significant when compared to the same-sex control. Treatment-related serum chemistry profiles also supported the liver as a target organ. AST and ALT activities were increased in 8,000 ppm males and recovery group males and females. Treatment-related increases in the serum levels of total protein occurred in the 8,000 ppm males, while albumin and globulin increases occurred in the 8,000 ppm and recovery males. Gross findings included liver masses that occurred with greatest frequency at 4,000 and 8,000 ppm, including the 8,000 ppm recovery group; these masses corresponded to hepatocellular neoplasms or involvement by lymphoma or histiocytic sarcoma.

The incidences of cytoplasmic eosinophilia, diffuse slight to moderate hepatocellular enlargement, and slight to moderate pigment were significantly increased in 8,000 ppm males and females relative to the control ([ REF _Ref99635974 \h * MERGEFORMAT ]). These non-neoplastic changes were observed in the 8,000 ppm recovery group at lower incidences than in the 8,000 ppm group. With the exception of pigment in females, the incidences in the recovery group were significantly greater than the control incidences. Very slight focal necrosis was present in one control female, two 500 ppm females, four 8,000 ppm females, and three 8,000 ppm males. Very slight individual cell necrosis was observed in four 8,000 ppm males.

| **Table [ SEQ Table * ARABIC ]. Incidence of Selected Non-neoplastic Lesions in B6C3F1 Mice Exposed to DINP in the Diet for Two Years (Moore 1998b)** | |
|---|---|
| **Lesion** | **Dose Group mg/kg/day (ppm)** |

| | Control | 90 M<br>112 F<br>(500) | 276 M<br>336 F<br>(1,500) | 742 M<br>910 F<br>(4,000) | 1,560 M<br>1,888 F<br>(8,000) | Recovery (b)<br>1,560 M<br>1,888 F<br>(8,000) |
|---|---|---|---|---|---|---|
| Males | | | | | | |
| Diffuse hepatocellular enlargement | 0/55 (a)<br>(0%) | 1/50<br>(2.0%) | 1/50<br>(2.0%) | 2/50<br>(4.0%) | 45/55*<br>(81.8%) | 10/50*<br>(20.0%) |
| Increased cytoplasmic eosinophilia | 0/55<br>(0%) | 0/50<br>(0%) | 0/50<br>(0%) | 0/50<br>(0%) | 52/55*<br>(94.5%) | 10/50*<br>(20.0%) |
| Pigment | 0/55<br>(0%) | 0/50<br>(0%) | 0/50<br>(0%) | 0/50<br>(0%) | 49/55*<br>(89.1%) | 6/50*<br>(12.0%) |
| Females | | | | | | |
| Diffuse hepatocellular enlargement | 0/55<br>(0%) | 0/51<br>(0%) | 0/50<br>(0%) | 1/50<br>(2.0%) | 52/55*<br>(94.5%) | 6/50*<br>(12.0%) |
| Increased cytoplasmic eosinophilia | 0/55<br>(0%) | 0/51<br>(0%) | 0/50<br>(0%) | 0/50<br>(0%) | 53/55*<br>(81.8%) | 6/50*<br>(12.0%) |
| Pigment | 1/55<br>(1.8%) | 1/51<br>(2.0%) | 2/50<br>(4.0%) | 2/50<br>(4.0%) | 41/55*<br>(74.5%) | 3/50<br>(6.0%) |

Source: Tables 11A and 11C in Moore (1998b). Abbreviations: M = male; F = female

* = significantly different from control (p#0.05) by Fisher's Exact test performed by Syracuse Research Corporation.

(a) Number of animals with lesion/total number of animals examined; percent incidence of lesion in parentheses.

Incidences are sum of unscheduled deaths and lesions observed at terminal sacrifice

(b) The 8,000 ppm recovery group received 8,000 ppm for 78 weeks, followed by a 26-week recovery period during which the test animals received basal diet alone.

After 78 and 104 weeks of treatment, the mean liver palmitoyl-CoA oxidase activity (an indicator of peroxisome proliferation) in hepatic microsomes was significantly increased in male and female mice at 8,000 ppm when compared to the controls. Liver mean protein concentration was significantly increased in 8,000 ppm mice at both time points. These results indicate that treatment with 8,000 ppm DINP induced peroxisome proliferation in exposed animals. Neither a NOAEL nor reliable LOAEL could be determined for this effect because measurements were not made in the 500, 1,500, or 4,000 ppm dose groups. DINP exposure did not induce significant increases in mean labeling index at 78 or 105 weeks, suggesting that sustained cellular proliferation did not occur in treated mice under the conditions used in this study.

DINP-induced liver toxicity was partially reversed in the 8,000 ppm recovery groups. Values for liver weight were comparable to control values and histological evidence of liver enlargement was not observed in the male or female recovery groups. The incidences of non-neoplastic lesions in the recovery groups were decreased at study termination relative to the 8,000 ppm groups, but in most cases were significantly greater than the control values.

2. Kidney Effects

Treatment-related effects on kidney weights in male mice were seen at the interim and terminal sacrifices. At 79 weeks, mean absolute kidney weights were decreased in 1,500, 4,000, and 8,000 ppm males. Kidney-to-body and kidney-to-brain weights were reduced in 4,000 and 8,000 ppm males. At study termination, absolute and relative-to-brain weight means were significantly reduced in 1,500, 4,000, and 8,000 ppm males and in the male recovery group, but there was no correlating histopathological finding. The mean value for kidney-to-body weight was significantly reduced in 4,000 ppm males, but not for males in other dose groups. No significant effect on relative or absolute kidney weight was observed in females. Urinalysis findings in 8,000 ppm males and females at 26, 52, 78, and 104 weeks included (1) significant increases in urine output; (2) significant decreases in mean urine osmolarity; and (3) significantly decreased sodium, potassium, and chloride levels in high-dose mice. The study authors concluded that there was not DINP-related change in glomerular filtration rate; however, this pattern may suggest a compromised ability to concentrate urine in the renal tubule epithelium. The study authors further suggested that this effect may have resulted from exacerbation of chronic progressive nephropathy (histologically evident in 8,000 ppm females only). At necropsy, the kidneys of 8,000 ppm females had a granular pitted/rough appearance. This finding corresponded with increased incidence and severity of chronic progressive nephropathy in the 8,000 ppm females (control incidence: 40/60, mean severity 0.8; 4,000 ppm: 39/60, 0.8; 8,000 ppm, 61/62, 1.8; recovery: 39/50, 0.9). The incidence of this lesion in females was significantly ($p \leq 0.05$) increased at 8,000 ppm when compared to the incidence in the control group.

DINP-induced kidney toxicity was only partially reversed in the 8,000 ppm recovery groups. The reversibility of the kidney effects in the 8,000 ppm recovery groups was not

as pronounced as that for liver effects. Kidney weights in males were partially reversed upon cessation of DINP exposure. The incidence and severity of chronic progressive nephropathy in female mice were comparable to those of the control group upon termination, suggesting that neuropathy is reversible or that exacerbation of this lesion halted when exposure to DINP was discontinued.

3. Carcinogenicity

Moore (1998b) reported the results of statistical analyses on tumor incidence data for all deaths (i.e., the sum of tumor incidences from unscheduled deaths, interim sacrifice, and terminal sacrifice). In males, significant positive trends were observed for the incidences of hepatocellular carcinoma and hepatocellular carcinoma and adenoma combined. The incidences of hepatocellular carcinoma and hepatocellular carcinoma and adenoma combined were significantly increased when compared to the control. In females, significant positive trends were observed for the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular carcinoma and adenoma combined. The incidences of hepatocellular adenoma in the 8,000 ppm and recovery groups were significantly greater than the control values. The incidences of hepatocellular carcinoma were significantly increased in females in the 4,000 and 8000 ppm and recovery groups. The incidences of hepatocellular carcinoma and adenoma combined were increased in the 1,500, 4,000 and 8,000 ppm groups and in the recovery group.

CHAP (2001) reported a more extensive statistical analysis of the tumor data obtained in the Moore (1998b) study. CHAP extracted individual animal data from the original study report, which were subsequently analyzed by NTP using six statistical procedures (Life Table, Poly 3, Poly 1.5, Poly 6, Logistic Regression, and Cochrane-Armitage/Fisher's Exact). The selection of results from a particular procedure depended, in part, on whether the lesion of interest was lethal or nonlethal. Results from the CPSC/NTP analysis for selected tumors are shown in [ REF _Ref99637154 \h * MERGEFORMAT ]. The results for trend and individual comparisons of dose groups to the control for hepatocellular tumors showed the same pattern as reported by Moore (1998b), with the exception that NTP did not analyze data for the recovery groups. No tumors originating in the kidney were reported.

| Table [ SEQ Table * ARABIC ]. Overall Incidence of Hepatocellular Tumors in B6C3F1 Mice (Moore 1998b) | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Dose Group mg/kg/day (ppm)** | | | | | |
| | **Control** | **90 M 112 F (500)** | **276 M 336 F (1,500)** | **742 M 910 F (4,000)** | **1,560 M 1,888 F (8,000)** | **Recovery (a) 1,560 M 1,888 F (8,000)** |
| Males | | | | | | |
| Survival | 87 | 87 | 76 | 79 | 63 | 81 |
| Hepatocellular adenoma | 10/70 (b) (14%) | 7/67 (10%) | 8/66 (12%) | 15/65 (23%) | 13/70 (19%) | – (c) |
| Hepatocellular carcinoma | 10/70 (14%) | 8/67 (12%) | 10/66 (15%) | 17/65 (26%) | 20/70* (29%) | – |
| Hepatocellular adenoma and carcinoma (combined) | 16/70 (23%) | 13/67 (19%) | 18/66 (27%) | 28/65* (43%) | 31/70* (44%) | – |
| Females | | | | | | |
| Survival | 81 | 79 | 81 | 62 | 77 | 75 |
| Hepatocellular adenoma | 2/70(3%) | 4/68(6%) | 5/68(7%) | 4/67(6%) | 18/70* 26%) | – |
| Hepatocellular carcinoma | 1/70(1%) | 2/68(3%) | 5/68(7%) | 7/67* (10%) | 19/70* (27%) | – |
| Hepatocellular Adenoma and carcinoma (combined) | 3/70(4%) | 5/68(7%) | 10/68* (15%) | 11/67* (16%) | 33/70* (47%) | – |

Source: CHAP (2001) Table IX-6 (page 73) and Appendix B.
Abbreviations: M = male; F = female
* = significant from the control at $p<0.05$ by logistic regression analysis
(a) The high dose/recovery group received 8000 ppm for 78 weeks, followed by a 26-week recovery periodduring which they received basal diet alone.
(b) Number of animals with tumor/total number of animals examined. Percent tumor incidence in parentheses.
(c) Incidence data for the recovery groups were not analyzed by CPSC/NTP.

4. Conclusions for Moore (1998b)

NOAEL values for systemic toxicity of 1,500 ppm for male mice and 500 ppm for female mice were identified in Moore (1998b) based on non-cancer and cancer effects. The value for females was based on a statistically significant increase in the combined incidence of hepatocellular adenoma and carcinoma at 1,500 ppm. The value for males was based on a significant increase in the incidence of liver neoplasms at 4,000 ppm. In reviewing the study

data, EPA has determined that other potentially adverse effects occurred at the 1,500 ppm level. These effects include decreased absolute and relative kidney weights throughout the study, increased incidence of liver masses in males, and increased absolute liver weights and significantly decreased mean body weight gain in females. Based on these effects, EPA has concluded that the LOAEL for chronic systemic toxicity in this study is 1,500 ppm (276 and 336 mg/kg/day for males and females, respectively).

d) Chronic Toxicity and Carcinogenicity in Rats-Biodynamics-1986

The chronic toxicity and carcinogenicity of DINP were investigated in a non-standard guideline and GLP-compliant 2-year feeding study in CD Sprague-Dawley rats. Rats (60/sex/group) were administered the test substance via the diet at 0 (feed only), 500, 5,000, or 10,000 ppm (equivalent to 0, 27, 271, or 553 mg/kg-bw/day, respectively, in males and 0, 33, 331, or 672 mg/kg-bw/day, respectively, in females) for 24 months (732–740 consecutive days, depending on day of sacrifice). Additional animals (10/sex/group) were administered the test substance concurrently at the same concentrations and were sacrificed immediately after 12 months of exposure for an interim timepoint. Concentrations were selected based on subchronic toxicity data. Morbidity and mortality were assessed twice daily and detailed physical examinations were conducted weekly. Body weights and food consumption were measured weekly for the first 14 weeks, every other week from weeks 16 through 26, and monthly thereafter, including at both the interim and terminal sacrifices. Ophthalmoscopic examinations were conducted at months 0 (pre-test), 6, 12, 18, and 24. Blood and urine for hematology, clinical chemistry, and urinalysis were collected at months 6, 12, 18, and 24 from 10 randomly selected animals/sex/group; the same animals were used at all intervals when feasible.

Animals were sacrificed at 12 or 24 months. At sacrifice, animals received gross necropsy and selected organs were weighed. Histopathological examinations were performed on selected tissues in control and high-dose animals. Only the livers were examined in low- and mid-dose animals. Effects of treatment were considered statistically significant at a level of $p < 0.05$. Body weights, food consumption, hematology, clinical chemistry, and organ weights (all of the endpoints analyzed statistically) were analyzed by Bartlett's test for homogeneity of variance. Data with homogenous variance were analyzed by one-way ANOVA followed by Dunnett's test if the ANOVA was significant. Data with heterogenous

variance were analyzed by Kruskal-Wallis test followed by Dunn's test if the Kruskal-Wallis test was significant. To test dose-dependency, parametric data were analyzed for trend and fit with standard regression techniques and nonparametric data were analyzed for trend with Jonckheere's test.

1. Mortality, Clinical Observations, and Ophthalmology

No treatment-related differences in mortality were observed. Clinical signs were similar across all groups. Additionally, no treatment-related changes in ophthalmology were observed.

2. Body Weight, Body Weight Gain, and Food Consumption

No treatment-related changes in body weight or food consumption were observed in males of any treatment group. In females of the high-dose group, body weights were decreased relative to control throughout the study, generally reaching statistical significance from week 11 onward, and the magnitude of the change generally being ≥10% relative to control from week 78 onward, with terminal body weights decreased by 10% relative to control. Decreases in mean body weights of the high-dose females were attributed to the administration of the test substance. No treatment-related changes in body weight or food consumption were observed in females of the low- or mid-dose groups. In females of the high-dose group, food consumption was generally increased from week 10 onward, with the most pronounced differences occurring during weeks 10–12 (significantly increased by 5–8% relative to control) and weeks 74–94 (significantly increased by 9–12% relative to control). Although in most cases, these differences from control were small, the trend was considered indicative of a relationship to the administration of the test substance.

3. Hematology

In males of the high-dose group at month 24, hematocrit, hemoglobin, and erythrocytes (red blood cell [RBC]) counts were significantly decreased relative to control by 18, 19, and 17%, respectively. No treatment-related changes in hematological parameters were observed in males of the low- or mid-dose groups at any timepoints or in males of the high-dose group

prior to month 24. No treatment-related changes in hematological parameters were observed in females of any group at any timepoint. Other sporadic, some statistically significant, differences from control were noted in treated males and females throughout the study; however, there were no consistent dose-related trends evident in the data that were considered indicative of a treatment-related effect and the changes in high-dose males appear to be within the range of biological variation for this endpoint under the conditions of this study.

4. Clinical Chemistry

Changes in clinical chemistry endpoints reported below were not statistically significant relative to controls, unless specifically stated otherwise. At months 6, 12, 18, and 24, serum aspartate aminotransferase (AST or SGOT) was increased relative to controls in males of the mid-dose group by 35, 49, 44, and 11%, respectively, and in males of the high-dose group by 118, 103, 91, and 111%, respectively. At months 6, 12, 18, and 24, serum alanine aminotransferase (ALT or SGPT) was increased relative to controls in males of the mid-dose group by 66, 79, 70, and 6%, respectively, and in males of the high-dose group by 292, 203, 232 (statistically significant), and 218%, respectively. At months 6 and 12, serum alkaline phosphatase (ALP) was increased relative to controls in males of the mid-dose group by 35 and 47%, respectively, and significantly increased relative to controls in males of the high-dose group by 88 and 76%, respectively. No treatment-related changes in serum ALP were observed in males at months 18 or 24. In females, serum AST and ALT were increased relative to control in the high-dose group by 63 and 89%, respectively, at month 6; no treatment-related changes in serum AST or ALT were observed at subsequent timepoints.

Serum ALP was increased relative to control in females of the high-dose group by 81 (statistically significant) and 38%, respectively, at months 18 and 24; no treatment-related changes in serum ALP were observed at previous timepoints in this group or in females of the low- or mid-dose groups. The increased serum AST, ALT, and ALP in treated males were for the most part not statistically significant; however, these findings were considered treatment-related due to the consistency with which they were noted in the treated males at most timepoints. The increased AST and ALT values in females of the high-dose group at month 6 were attributed to markedly increased values in one animal (animal no. 4,570) only; the increases were not considered to be related to the administration of the test substance due to their isolated occurrence in only one animal at only one timepoint and was determined to be

statistical outliers via the Grubb's outlier test, conducted. The increased ALP in females of the high-dose group at month 18 and month 24 is treatment-related and adverse.

5. Urinalysis

No treatment-related changes in urinalysis parameters were observed in males or females of any group at any timepoint.

6. Gross Necropsy

The animals necropsied at the 12-month interim sacrifice did not exhibit any detectable treatment-related gross morphologic changes. At necropsies conducted after the interim sacrifice, numerous changes—most of which are commonly observed in the laboratory rat of the same strain and similar age—were recorded. Because they occurred either sporadically or with a similar frequency in both treated and untreated rats, they did not appear to be related to treatment. Similarly, no treatment-related changes were observed upon gross necropsy in males or females of any group at the interim or the terminal sacrifice.

7. Organ Weights

Absolute and relative liver weights were significantly increased in high-dose males at both the interim (30 and 34%, respectively) and terminal (27 and 27%) sacrifices, while liver weights in lower-dose male groups were similar to controls. Absolute and relative kidney weights were also significantly increased in high-dose males at both the interim (19 and 25%, respectively) and terminal (13 and 12%) sacrifices; nonsignificant increases of 11% were seen in the mid-dose group at interim sacrifice only. Thyroid weights were increased relative to controls in all male treated groups at the interim sacrifice, but the change did not increase with dose and was not always statistically significant (absolute: 28, 24 and 15%; relative: 26*, 26* and 20% in the low-, mid-, and high-dose groups, respectively; * = statistically significant). At terminal sacrifice, absolute and relative thyroid weights were increased in the low-dose males (44 and 38%, respectively), but decreased relative to controls in the mid- and high-dose males (−11 to −19%); however, these changes were not statistically significant.

In females, absolute and relative liver weights were significantly increased in the high-dose group at both interim (26 and 36%) and terminal (14 and 25%) sacrifices. In the mid-

dose females, there were non-significant increases in absolute (14%) and relative (11%) liver weight at interim sacrifice and absolute liver weight (15%) at terminal sacrifice, and a significant increase in relative liver weight (16%) at terminal sacrifice. In high-dose females, relative kidney weights were significantly increased by 20% at interim sacrifice and 14% at terminal sacrifice, while absolute kidney weights showed only nonsignificant 11 and 3% increases, respectively. The mid-dose females showed no change from controls at the interim sacrifice, but small increases of 9% (statistically significant) in absolute kidney weight and 10% (non-significant) in relative kidney weight at the terminal sacrifice. Thyroid weights were significantly increased relative to controls in all female treated groups at the interim sacrifice (absolute: 107, 102 and 111%; relative: 93, 93 and 129% in the low-, mid-, and high-dose groups, respectively). At terminal sacrifice, however, the only changes in thyroid weight were nonsignificant increases of 14 and 21% for absolute and relative weight in mid-dose females and 16% for relative weight in high-dose females.

Increases noted in liver, kidney, and absolute and relative organ weights were attributed to the administration of DINP.

8. Histopathology

Incidence data for select histopathological observations are shown in [ REF _Ref99636491 \h * MERGEFORMAT ]. Lesions potentially related to treatment were seen in the liver (minimal-to-mild focal necrosis, spongiosis hepatis, neoplastic nodules, hepatocellular carcinoma), kidney (medullary mineral deposits), pancreas (islet cell adenomas and carcinomas), parathyroid (hyperplasia), testes (interstitial cell hyperplasia and tumors) and uterus (endometrial hyperplasia). The results of statistical analyses performed for this review are included in [ REF _Ref99636491 \h * MERGEFORMAT ]. Statistically significant findings in the liver were a dose-related trend for increased incidence of focal necrosis (minimal-to-mild severity) in males, with significant pair-wise increases in the low- and high-dose groups. There were also significant trends for increased incidence of spongiosis hepatis in both males and females, with pair-wise increases in the mid- and high-dose males. Females showed a significant trend for hepatocellular carcinomas, with a significant increase in the high-dose group. Significant findings in other tissues were increased incidence of medullary mineral deposits in the kidney in high-dose males, increased incidence of interstitial cell hyperplasia in the testes in high-dose males and increased

incidence of endometrial hyperplasia in the uterus of high-dose females. The study provided comparisons to historical controls from the laboratory for selected lesions in the liver and testes. They reported that the incidence of hepatocellular carcinomas in mid- and high-dose males and females exceeded the range of historical controls, while the incidence of neoplastic nodules in all groups was well within the historical control range. In the testes, the incidence of interstitial cell tumors (data for high-dose males only) was within the historical range. The incidence of interstitial cell hyperplasia in the high-dose males, however, exceeded the historical control range.

The microscopic evaluation of the liver in all treated groups and other select tissues in the high-dose only suggested the following compound-related changes: (1) hepatocellular carcinomas in mid- and high-dose males and females, (2) minimal to slight focal hepatocellular necrosis in the treated males, (3) testicular interstitial cell hyperplasia in the high-dose males, and (4) renal medullary mineral deposit in the high-dose males. In addition, slightly increased incidences of pancreatic islet cell tumors and parathyroid gland hyperplasia were observed in high-dose males whereas endometrial hyperplasia was observed in high-dose females; the significance of these findings is uncertain.

Hepatic necrosis in males at all dose levels is treatment-related and adverse and considers the significant increases in incidence of spongiosis hepatis in mid- and high-dose males and high-dose females to be treatment-related and adverse. The incidences of renal medullary mineral deposits and testicular interstitial cell hyperplasia in high-dose males are treatment-related and adverse, and also considers the significant increase in incidence of uterine endometrial hyperplasia in high-dose females to be treatment-related and adverse. Parathyroid gland hyperplasia in high-dose males was not considered treatment-related, as statistical tests showed no difference from control. For tumors, the hepatocellular carcinomas are treatment-related and adverse in females and potentially in males, although incidence in males is not dose-related, not significantly greater than concurrent controls, and only slightly greater than historical controls. The findings for testicular interstitial cell tumors to be equivocal; incidence was higher in high-dose males than controls but was not statistically significant and was within the range of historical controls. It is noted that interstitial cell hyperplasia in the testes, potentially a related pre-neoplastic lesion, was increased in high-dose males. The nonsignificant incidences of neoplastic nodules in the liver and pancreatic tumors to be within the range of normal biological variation. It is noted that although effects

were observed upon histopathological evaluation of the kidneys, testes, and uterus, no histopathological evaluation was conducted on samples from the low- or mid-dose groups, which limits the assessment of dose-dependency and effect levels. Remaining findings appear to be unrelated to the administration of the material tested.

**Table [ SEQ Table * ARABIC ]. Histopathological Observations in Animals Exposed to DINP**

| Observation | | Dietary Concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Males | | | | Females | | | |
| | | **0** | **500** | **5,000** | **10,000** | **0** | **500** | **5,000** | **10,000** |
| Liver[2] | | | | | | | | | |
| No. Examined | | 70 | 69 | 69 | 70 | 70 | 70 | 70 | 70 |
| Hepatocellular carcinoma | – | 2 | 2 | 6 | 4 | 0† | 0 | 5 | 7* |
| Neoplastic nodule(s) | – | 2 | 5 | 6 | 5 | 1 | 1 | 5 | 2 |
| Spongiosis hepatitis | – | 16† | 11 | 30* | 32* | 4† | 3 | 6 | 11 |
| Focal necrosis (minimal-to-mild) | – | 5† | 17* | 11 | 23* | 10 | 15 | 7 | 10 |
| Kidneys[3a] | | | | | | | | | |
| No. Examined | | 70 | 0 | 0 | 70 | 70 | 0 | 0 | 70 |
| Renal medullary mineral deposit | Total | 3 | – | – | 25* | 14 | – | – | 15 |
| | Unilateral | 3 | – | – | 9 | 6 | – | – | 4 |
| | Bilateral | 0 | – | – | 16 | 8 | – | – | 11 |
| Pancreas[4] | | | | | | | | | |
| No. Examined | | 70 | 0 | 0 | 70 | 69 | 0 | 0 | 70 |
| Pancreatic islet cell adenoma | – | 6 | – | – | 8 | 1 | – | – | 1 |
| Pancreatic islet cell carcinoma | – | 1 | – | – | 4 | 0 | – | – | 0 |
| Parathyroid[5a] | | | | | | | | | |
| No. Examined | | 56 | 0 | 0 | 62 | 57 | 0 | 0 | 58 |
| Parathyroid gland hyperplasia | Total | 19 | – | – | 29 | 17 | – | – | 15 |
| | Unilateral | 7 | – | – | 9 | 7 | – | – | 8 |
| | Bilateral | 12 | – | – | 20 | 10 | – | – | 7 |
| Testes[6a] | | | | | | | | | |
| No. Examined | | 69 | 0 | 0 | 70 | N/A | N/A | N/A | N/A |
| Interstitial cell hyperplasia | Total | 4 | – | – | 22* | – | – | – | – |
| | Unilateral | 3 | – | – | 9 | – | – | – | – |
| | Bilateral | 1 | – | – | 13 | – | – | – | – |
| Interstitial cell tumors | Total | 2 | – | – | 7 | – | – | – | – |
| | Unilateral | 2 | – | – | 6 | – | – | – | – |
| | Bilateral | 0 | – | – | 1 | – | – | – | – |
| Uterus[7] | | | | | | | | | |
| No. Examined | | N/A | N/A | N/A | N/A | 70 | 0 | 0 | 70 |
| Endometrial hyperplasia | – | – | – | – | – | 2 | – | – | 13* |

[1] Data in this table indicate all animals assessed for histopathology throughout the study; i.e. including the interim sacrifice, the terminal sacrifice, and unscheduled deaths. For late-developing tumors (hepatocellular carcinoma, pancreatic islet cell tumors, testicular interstitial cell tumors), statistical analysis was performed excluding animals that died or were sacrificed up to 12 months, leaving n = 57, 57, 59, 59 in males and n = 59, 56, 60, 59 in females in the control, low-, mid- and high-dose groups, respectively.
[2] Data from Appendix K, Figure 1, pp. 11 (pp. 426 of the study report PDF)
[3] Data from Appendix K, Figure 6, pp. 18 (pp. 433 of the study report PDF)

| Table [ SEQ Table * ARABIC ]. Histopathological Observations in Animals Exposed to DINP | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Dietary Concentration (ppm) | | | | | | | |
| | Males | | | | Females | | | |
| **Observation** | **0** | **500** | **5,000** | **10,000** | **0** | **500** | **5,000** | **10,000** |
| [4] Data from Appendix K, Figure 8, pp. 22 (pp. 437 of the study report PDF) | | | | | | | | |
| [5] Data from Appendix K, Figure 7, pp. 20 (pp. 435 of the study report PDF) | | | | | | | | |
| [6] Data from Appendix K, Figure 3, pp. 14 (pp. 429 of the study report PDF) | | | | | | | | |
| [7] Data from Appendix K, Figure 5, pp. 17 (pp. 432 of the study report PDF) | | | | | | | | |
| * $p < 0.05$ based on a two-tailed Fisher's exact test calculated for this review. | | | | | | | | |
| † Statistically significant trend ($p < 0.05$) based on a Chi-square contingency trend test calculated for this review. | | | | | | | | |
| [a] Observations that were specified to be unilateral or bilateral were pooled and total incidences were analyzed statistically. | | | | | | | | |

9. Conclusions for the Biodynamics study

Under the conditions of this study, the test substance was carcinogenic, producing hepatocellular carcinomas in male and female Sprague-Dawley CD rats after exposure to 5,000 or 10,000 ppm in the diet. Although treatment-related effects also occurred at the 500-ppm level, there was no increase in tumors at this level.

The LOAEL for non-cancer systemic effects in males is 500 ppm based on increased incidence of hepatic necrosis; a NOAEL was not established. Other adverse systemic effects in males at higher doses included (1) increased serum AST, ALT, and ALP; (2) increased liver and kidney weights; (3) increased incidences of spongiosis hepatis in the liver; (4) medullary mineral deposits in the kidney; and (5) interstitial cell hyperplasia in the testes. The LOAEL for non-cancer systemic effects in females is 5,000 ppm based on increased liver weight; the NOAEL is 500 ppm. Other effects in females at higher doses were decreased body weight and increased food consumption, increased serum ALP, spongiosis hepatis in the liver, and endometrial hyperplasia in the uterus.

Study Limitations: Although effects were observed upon histopathological evaluation of the kidneys, testes, and uterus, no histopathological evaluation was conducted on samples from the low- or mid-dose groups, which limits the assessment of dose-dependency and effect levels.

e) Pathology Working Group Review of Data for Spongiosis Hepatis and MNCL (EPL 1999)

A Histopathology Peer Review and a Pathology Working Group Review (PWG) were conducted on selected lesions of the liver and spleen observed in Fischer-344 rats in the 2-year bioassays reported by Lington et al. (1997) and Moore (1998a). The PWG review evaluated the significance of spongiosis hepatis, foci of cellular alteration, primary

hepatocellular neoplasms in the liver, and the significance of mononuclear cell leukemia. The peer and PWG reviews were conducted in accordance with EPA Pesticide Regulation Notice 94-5, which describes the procedure to be followed for submission of pathology re-reads to the Agency.

1. Spongiosis Hepatis

Induction of spongiosis hepatis, also referred to as cystic degeneration by some authors, is of interest because it appears to be the most sensitive non-neoplastic response in rats chronically exposed to DINP (Lington et al. 1997, Moore 1998a). However, questions have arisen regarding the relationship of this lesion to other pathological processes occurring in animals treated with DINP that may not be relevant to humans, including peroxisome proliferation and MNCL. Although a few differences were noted, the Histology Peer Review and the PWG Review of lesions in the liver and spleen generally confirm the incidence data reported by the study pathologists. The incidences of spongiosis hepatis in the Lington et al. (1997) and Moore (1998a) studies as determined by the PWG are shown in [ REF _Ref99636665 \h * MERGEFORMAT ] and [ REF _Ref99636986 \h * MERGEFORMAT ].

The PWG noted that spongiosis hepatis might be found as an independent lesion or within foci of cellular alteration or hepatocellular neoplasms. In the reviewed studies, spongiosis hepatis was diagnosed whenever it occurred, regardless of relationship to other hepatic changes which were also present. This method of diagnosis differs from some standard pathology guidelines, which recommend that spongiosis hepatis not be diagnosed separately when it occurs within foci or tumors. The PWG concluded that the method of diagnosis used in the DINP rat studies made interpretation of spongiosis hepatis as a treatment-related effect difficult. As noted in EPL (1999), some differences were noted in the pathology protocols for the two studies which may have affected the reported incidences. These differences include the number of sections taken from the liver in each study and the protocol for examination of the spleen. These differences make the direct comparison of the results from Lington et al. (1997) and Moore (1998a) difficult and may account for the greater incidence of foci of cellular alteration and foci of spongiosis hepatis observed in the Lington et al. (1997) study.

**Table [ SEQ Table * ARABIC ]. Incidence of Mononuclear Cell Leukemia and Selected Hepatic Lesions at Terminal Sacrifice (104 Weeks) in the Lington et al. (1997) Study in Fischer-344 Rats as Determined by the Pathology Working Group (EPL 1999)**

| Lesion | Dose Group mg/kg/day (ppm) | | | |
|---|---|---|---|---|
| | Control | 15 M/18 F (300) | 152 M/184 F (3,000) | 307 M/375 F (6,000) |
| Males | | | | |
| MNCL | 32/81 | 27/80 | 48/80 | 49/80 |
| Hepatocellular adenoma | 3/81 | 1/80 | 2/80 | 1/80 |
| Hepatocellular carcinoma | 0/81 | 1/80 | 0/80 | 3/80 |
| Eosinophilic foci | 58/81 | 50/80 | 46/80 | 52/80 |
| Basophilic foci | 53/81 | 62/80 | 48/80 | 42/80 |
| Spongiosis hepatis | 22/81 | 24/80 | 51/80 | 62/80 |
| Females | | | | |
| MNCL | 22/81 | 21/81 | 29/80 | 41/80 |
| Hepatocellular adenoma | 0/81 | 4/81 | 0/80 | 2/80 |
| Hepatocellular carcinoma | 1/81 | 0/81 | 0/80 | 1/80 |
| Eosinophilic foci | 59/81 | 47/81 | 42/80 | 32/80 |
| Basophilic foci | 72/81 | 64/81 | 64/80 | 55/80 |
| Spongiosis hepatis | 4/81 | 1/81 | 3/80 | 4/80 |

Source: Modified from data in Table 6 in EPL (1999)
Abbreviations: M = male, F = female

**Table [ SEQ Table * ARABIC ]. Incidence of Mononuclear Cell Leukemia and Selected Hepatic Lesions at Terminal Sacrifice (104 Weeks) in the Moore (1998a) Study in Fischer-344 Rats as Determined bythe Pathology Working Group (EPL 1999)**

| Lesion | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 29.2 M 36.4 F (500) | 88.3 M 108.6 F (1,500) | 359 M 442 F (6,000) | 733 M 885 F (12,000) | Recovery (c) 637 M/773.6 F (12,000) |
| Males | | | | | | |
| MNCL | 21/55 | 23/50 | 21/50 | 32/55 | 28/55 | 30/50 |
| Hepatocellular adenoma | 2/55 | 4/50 | 1/50 | 4/55 | 7/55 | 6/50 |
| Hepatocellular carcinoma | 1/55 | 0/50 | 0/50 | 3/55 | 11/55 | 3/50 |
| Eosinophilic foci | 22/55 | 14/50 | 16/50 | 15/55 | 10/55 | 12/50 |

**Table [ SEQ Table * ARABIC ]. Incidence of Mononuclear Cell Leukemia and Selected Hepatic Lesions at Terminal Sacrifice (104 Weeks) in the Moore (1998a) Study in Fischer-344 Rats as Determined bythe Pathology Working Group (EPL 1999)**

| Lesion | Dose Group mg/kg/day (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 29.2 M 36.4 F (500) | 88.3 M 108.6 F (1,500) | 359 M 442 F (6,000) | 733 M 885 F (12,000) | Recovery (c) 637 M/773.6 F (12,000) |
| Basophilic foci | 40/55 | 34/50 | 33/50 | 28/55 | 27/55 | 25/50 |
| Spongiosis hepatis | 6/55 | 6/50 | 3/50 | 18/55 | 26/55 | 10/50 |
| Females | | | | | | |
| MNCL | 17/55 | 16/50 | 9/50 | 28/55 | 28/55 | 24/50 |
| Hepatocellular adenoma | 1/55 | 1/50 | 0/50 | 1/55 | 1/55 | 1/50 |
| Hepatocellular carcinoma | 0/55 | 0/50 | 0/50 | 1/55 | 6/55 | 2/50 |
| Eosinophilic foci | 10/55 | 5/50 | 7/50 | 7/55 | 0/55 | 4/50 |
| Basophilic foci | 37/55 | 32/50 | 31/50 | 18/55 | 5/55 | 13/50 |
| Spongiosis hepatis | 0/55 | 0/50 | 0/50 | 1/55 | 2/55 | 0/50 |

Source: Modified from data in Tables 9 and 10 in EPL (1999)
Abbreviations: M = male, F = female

2. MNCL

It has been suggested that the occurrence of spongiosis hepatis in rats exposed to DINP is a consequence of MNCL (EPL 1999). To address this possibility, the PWG examined the co-occurrence of spongiosis hepatis and MNCL in the Lington et al. (1999) and Moore (1998a) studies. A comparison of the numbers of animals with spongiosis hepatis and with and without MNCL diagnosed by the study pathologist did not support the conclusion that spongiosis hepatis is a consequence of MNCL as shown in [ REF _Ref99636986 \h * MERGEFORMAT ]. Approximately half of the rats with spongiosis hepatis also had MNCL. However, spongiosis hepatis was also observed in the absence of MNCL in the remainder of the affected animals.

3. Conclusions for EPL (1999)

The results of the Histopathology Peer Review and PWG generally confirmed the

original findings of the study pathologist(s). The PWG concluded that when data from both studies were considered, the NOAEL for DINP is 1,500 ppm (88 mg/kg/day) in male rats, based on the increased incidence of hepatic spongiosis in males at concentrations of 3,000 ppm (152 mg/kg/day) and greater. The PWG identified a NOAEL of 3,000 ppm (184 mg/kg/day) in females, based on increased incidence of MNCL at concentrations of 6,000 ppm (375 mg/kg/day) or higher.

4. Conclusions on Chronic Toxicity

i. Liver Effects

Adverse liver effects were noted in rats following chronic DINP exposure in three independent studies. Spongiosis hepatis, also called cystic or microcystic degeneration, has been identified as the most sensitive non-neoplastic[2] response resulting from DINP exposure and is thus considered the critical noncancer effect. The incidence of spongiosis hepatis was dose-related, and significantly elevated in male rats chronically treated with DINP in three studies conducted by different laboratories (Lington et al. 1997, Moore 1998a). The incidence of spongiosis hepatis was not elevated in female rats or mice, and in male rats it did not appear to increase in severity with increasing dose (CHAP 2001). In the Lington et al. (1997) study, the LOAEL for spongiosis hepatis was 152 mg/kg/day, while the LOAEL in the Moore (1998a) study was 359 mg/kg/day; the NOAELs were 15 and 88 mg/kg/day, respectively. A Histopathology Peer Review and Pathology Working Group (EPL 1999) independently evaluated the liver slides from rats chronically treated with DINP (Lington et al. 1997, Moore 1998a) and confirmed that the incidence of spongiosis hepatis was increased in male rats in each study. At the time the Technical Review of DINP was

[2] There is some debate regarding whether spongiosis hepatis/cystic degeneration should be regarded as a non-neoplastic, preneoplastic, or benign neoplastic lesion. The essential elements of this debate are discussed in Karbe and Kerlin (2002, 2004) and Bannasch (2003). There is general agreement that spongiosis hepatis originates in Ito cells, which comprise 5–15% of the total number of resident hepatic cells and play a role in the maintenance and repair of the liver following a variety of insults (Stroebel et al., 1995; Bannasch and Zerban 1997; Bannasch andSchroder 2002; Karbe and Kerlin 2002). Following liver injury, Ito cells undergo activation and are transformed into a phenotype with functions of proliferation, fibrogenesis, and contractility. Spongiosis hepatis appears to develop from Ito cells that transform to fibroblast-like cells (Karbe and Kerlin 2002). Some authors, notably Bannasch and colleagues, regard spongiosis hepatis as a preneoplastic and/or neoplastic change, based on its proliferative properties and persistent increased cell turnover rate in stop experiments using liver carcinogens (e.g., Stroebel et al. 1995; Bannasch and Zerban 1997; Bannasch and Schroder 2002; Bannasch 2003). In contrast, Karbeand Kerlin (2002) have reviewed the evidence on occurrence of spongiosis hepatis in response to chemical exposureand have concluded that it is a non-neoplastic lesion. In making this determination, Karbe and Kerlin noted that spongiosis hepatis is induced by a wide range of dissimilar chemicals and that the histology of the lesion lacks neoplastic characteristics such as compression, infiltration or metastasis, bulging above the surface of the liver, or lesion size greater than 8 mm.

written in 2000, the Agency had not reviewed the EPL (1999) report or seen the CHAP (2001) report that discusses the EPL (1999) report conclusions.

There is general agreement that spongiosis hepatis develops from the perisinusoidal (Ito) cells of the liver. The Agency believes that the existing data support the conclusion that the increased incidence of spongiosis hepatis in dosed rats is clearly related to DINP treatment. In evaluating the data for hepatic spongiosis, the Agency considered (1) the possibility that occurrence of spongiosis hepatis and induction of peroxisome proliferation were related; (2) the possibility that the occurrence of spongiosis hepatis was a consequence of MNCL; and (3) the relationship of spongiosis hepatis to hepatocellular cancer; and 4) the human relevance of hepatis spongiosis.

EPA believes that the occurrence of spongiosis hepatis and peroxisome proliferation in the livers of rats exposed to DINP are likely to be unrelated. Although peroxisome proliferation appeared to occur in both sexes of rats and mice, the incidence of spongiosis hepatis was increased only in male rats. In addition, spongiosis hepatis occurred in control animals and in treated animals at doses that did not induce peroxisome proliferation. These data indicate that induction of peroxisome proliferation per se is not a prerequisite for induction of spongiosis hepatis.

EPA does not believe that the increased incidence of spongiosis hepatis observed in rats exposed to DINP is secondary to MNCL. This conclusion is based on the findings of the EPL (1999), which noted that only about 50% of the animals with spongiosis hepatis also had MNCL and that the incidence of spongiosis hepatis increased in some rats that did not show signs of MNCL.

Spongiosis hepatis may be associated with or located within foci of cellular alteration or hepatocellular neoplasms. This association has prompted questions regarding the relationship of this lesion to carcinogenic processes in the liver. The Agency considers the relationship between spongiosis hepatis and hepatic carcinogenesis to be uncertain. The Agency notes, however, that there does not appear to be strong correlation between the induction of spongiosis hepatis and the occurrence of hepatocellular neoplasms in rats treated with DINP. In addition, 4 of the 12 studies reviewed by Karbe and Kerlin (2002) reported spongiosis hepatis in the absence of hepatocellular neoplasms while a fifth study observed hepatocellular cancer in females only.

Spontaneous and induced spongiosis hepatis lesions have been observed in fish as

well as rats, but the existence of the lesion in humans and other species is less well supported (Karbe and Kerlin 2002). It is unknown whether human Ito cells are capable of developing spongiosis hepatis as observed in rats. In the absence of information that clearly indicates a species-specific MOA for development of spongiosis hepatis, the occurrence of this lesion in rats is assumed to be relevant to humans.

Based on the available data, EPA believes that the weight of evidence indicates that the spongiosis hepatis is a treatment-related lesion in rats treated with DINP and that the occurrence of this lesion in animals is relevant to human health. The Agency has identified NOAEL and LOAEL values of 15 and 152 mg/kg/day, respectively, for the Lington et al. (1997) study and 88 and 359 mg/kg/day, respectively, for the Moore (1998a) study based on indications of serious liver damage (i.e., a statistically significant increased incidence of spongiosis hepatis and increased liver weight and liver enzyme activities) in male rats chronically exposed to DINP for 2 years.

ii. Kidney Effects

The kidney is a target organ of DINP in chronic toxicity studies in rats and mice. In rats, increased relative kidney weights were seen in a 21-day ((BIBRA 1986) and three 2-year rodent studies of DINP (Lington et al. (1997), Moore (1998a and 1998b)). In the 2-year study conducted by Lington et al. (1997), exposure to dietary levels of 152 and 307 mg/kg/day increased relative kidney weights of both male and female rats. An increase in tubular cell pigment was also noted in the tubular epithelium of high-dose males at 18 months. In the 2-year study reported by Moore (1998a), increased relative kidney weights occurred in rats receiving dietary doses greater than 359 mg/kg/day for males and 442 mg/kg/day for females. Urinalysis findings from the chronic studies included significant increases in urine output and corresponding decreases in electrolyte levels in high-dose males, suggesting compromised ability to concentrate urine in the renal tubule epithelium. These effects occurred at the same dosages that produced changes in kidney weights. In the Moore (1998a) study, serum urea levels (a marker of kidney toxicity) were significantly increased in rats exposed to 359 mg/kg/day and higher during the second half of the study. Increases in urine volume and kidney lesions were observed in the recovery group exposed to 733 mg/kg/day.

In the Moore (1998a) study, male rats with increased kidney weights also had

increased mineralization of renal papillae. However, it is unlikely that the histological effects reported (mineralization of renal papillae in male rats and pigmentation of kidney tubule cells) account for the increased weights of the kidneys. EPA recognizes that in male rats, kidney tumors are induced by an alpha-2u-globulin mechanism that may not be relevant to humans.

The kidney was also a target organ for DINP toxicity in the chronic study in mice (Moore 1998b). Kidney weights were significantly decreased at doses of 1,500 ppm (276 mg/kg/day) and above in male mice. This decrease in kidney weight correlated with clinical chemistry findings of higher urine volumes accompanied by lower osmolarity (with lower concentrations of sodium, potassium and chlorides) in the highest dose group and recovery groups of both sexes. The urinalysis findings suggest compromised ability to concentrate urine in the renal tubule epithelium. Histopathology findings included a DINP-induced increase in the incidence of chronic progressive nephropathy in females of the highest dose group (but not in males).

Granular pitted/rough kidneys were observed in female mice receiving the 8,000 ppm diet (1,888 mg/kg/day) and corresponded to increased incidence/severity of treatment-related nephropathy. The recovery group had a decreased incidence of chronic progressive nephropathy, suggesting that the effects of DINP were partially reversible upon cessation of DINP treatment or that cessation of treatment prevented exacerbation of existing lesions.

Kidney changes in female mice (increased incidence and severity of nephrotoxicity) occurred at 8,000 ppm (1,888 mg/kg/day) and in male and female rats (increased kidney weights, compromised ability to concentrate urine) at 6,000 ppm (359 and 442 mg/kg/day, respectively). Such changes are indicative of kidney toxicity. Although effects in male rats appear to be due to alpha-2u-globulin nephropathy, the toxic kidney effects in female mice and increased kidney weights in female rats cannot be explained by an alpha-2u-globulin MOA.

5. Conclusions on Carcinogenicity

i. Liver Tumors

Dietary exposure to DINP induced liver tumors in male and female rats fed 12,000 ppm (Moore 1998a) in male mice fed 4,000 ppm and above, and in female mice fed 1,500 ppm and above (Moore 1998b) when tested in 2-year oral bioassays. An increased

incidence of liver carcinoma was also observed in male rats fed 6,000 ppm in the 2-year bioassay conducted by Lington et al. (1997), although the response did not reach statistical significance. EPA believes that these data are sufficient to indicate that DINP is a liver carcinogen in rats and mice. The MOA for induction of DINP-induced liver tumors in rodents appears to involve peroxisome proliferation based on biochemical evidence of dose-related peroxisome proliferation in subchronic and chronic studies. An important issue in evaluating the hazard of DINP is the relevance of carcinogenic effects in rodents to human health.

The induction of hepatic tumors in rodents by DINP might be related to its ability to induce peroxisome proliferation. Peroxisome proliferators are a structurally diverse group of non-mutagenic chemicals that induce a broad range of responses via interaction with peroxisome proliferator activated receptors (PPAR). Some in the scientific community believe that the liver tumors which develop in rats and mice chronically exposed to DINP are mechanistically related to activation of PPAR receptor subtype alpha (PPAR alpha) (Kaufmann et al. 2002). Transgenic mice that lack PPAR alpha are generally refractory to the pleiotropic effects of peroxisome proliferators, such as peroxisome proliferation, liver enlargement, and liver cancer (Lee et al. 1995; Valles et al. 2003). However, there have been no 2-year studies of DINP in transgenic mice that lack PPAR alpha to determine whether tumors develop in the liver.

The relevance of a PPAR alpha-mediated carcinogenic MOA to humans is unclear. There are no adequate epidemiological studies on cancer in humans exposed to PPAR alpha agonists. Although humans and nonhuman primates express functional PPAR alpha and hypolipidemic drugs are known to act through PPAR alpha in humans, *in vivo* studies of DINP in primates (e.g., Hall et al. 1999; Pugh et al. 2000) and *in vitro* studies of cultured primate or human cells (e.g., Benford et al. 1986; Shaw et al. 2002) exposed to DINP or its metabolite MINP suggest that humans are refractory to the induction of peroxisome proliferation. The basis for the species differences in these studies is unknown but may be related to differences in the quantity of PPAR alpha or to differences in the regulatory sequences of the rodent and primate genes (Shaw et al. 2002). On other hand, human and mouse adenoviral recombinant PPAR alpha expressed in PPAR alpha deficient mice fully restored the development of peroxisome proliferator-induced immediate pleiotropic responses, including peroxisome proliferation and enhanced expression of genes involved

in lipid metabolism, suggesting that the human PPAR alpha is functionally competent and is equally as dose-sensitive as mouse PPAR alpha in inducing peroxisome proliferation within the context of mouse liver environment (Yu et al. 2001)

New information has emerged on the mechanism(s) by which peroxisome proliferating chemicals produce certain carcinogenic responses in rodents, including advances in the understanding of the underlying genetic factors that mediate the biochemical and cellular responses to such chemicals (summarized in Klaunig et al. 2003; Corton et al. 2014 and 2018). To study the question of whether peroxisome proliferating chemicals such as DINP are a hazard to humans considering this new information, several panels and workshops have been convened and charged with reviewing the state of the science on the relationship between peroxisome proliferation and hepatocarcinogenesis in rodents and the human relevance of PPAR alpha-induced liver tumors. One of the first panels, composed of government, academic and industry scientists, concluded that significant quantitative differences in PPAR alpha-induced liver effects associated with hepatic tumor formation exist between humans and rodents (Corton et al. 2014). Based on quantitative differences between species, most panel members felt that the PPAR alpha mode of action for liver tumorigenesis is "not relevant to humans," however, several panel members concluded that it was more appropriate to conclude that the PPAR alpha mode of action is "unlikely to be relevant to humans." In a subsequent workshop sponsored by the Toxicology Forum, the human relevance of rodent PPAR alpha and CAR mediated modes of action for liver tumors were considered by industry, academic, and government experts (Felter et al. 2018). Similar to the first panel, most workshop participants concluded that the PPAR alpha and CAR modes of action are not relevant to humans based on qualitative and quantitative differences. In contrast, California OEHHA classified DINP as a carcinogen under Proposition 65 in part based on evidence that DINP can induce liver tumors in mice and rats (OEHHA 2013b). In considering the role of PPAR alpha in inducing liver tumors, California OEHHA (2013a) concluded that there was sufficient evidence to suggest that "PPAR alpha activation may not be causally related to DINP-induced liver tumors in rats and mice" and that other mechanisms may be involved. Similarly, Environment Canada and Health Canada concluded that the mechanisms of DINP-induced liver tumorigenesis have not been fully elucidated, but that there is sufficient evidence to suggest multiple mechanisms, including PPAR alpha-independent

mechanisms, may be involved (EC/HC 2015a). Based on this, Health Canada (2015b) concluded that "the carcinogenic potential these phthalates [including DINP] in humans remains unclear but is most likely not relevant at environmentally meaningful exposure levels." As a result of this scientific uncertainty, EPA reserves judgement on the human relevance of liver tumorigenesis and whether DINP can reasonably be anticipated to cause cancer in humans.

ii. Kidney Tumors

In the study conducted in rats and reported by Moore (1998a), renal tubule cell carcinoma was observed in 2/65 high-dose (12,000 ppm) males and 4/50 recovery males compared to 0/65 in the control group. The response in recovery males was statistically significant relative to the control group. In the Lington et al. (1997) study, renal tubule cell carcinoma was observed in 1/80 low-dose (300 ppm) males and 2/80 high-dose males (6,000 ppm). No preneoplastic or neoplastic lesions were observed in females. Treatment-related histopathologic changes in the kidneys of rats were consistent with male rat-specific alpha-2u-globulin nephropathy. Additional evidence for alpha-2u-globulin nephropathy was obtained in the retrospective evaluation of archived kidney tissue from the Lington et al. (1997) study conducted by Caldwell et al. (1999). The data obtained in these studies were evaluated against published criteria for evaluating male-specific nephropathy and its relevance to human health (USEPA 1991b; IARC 1995). The results of this evaluation indicate that: (1) all three EPA criteria[3] for existence of the alpha-2u-globulin MOA have been met; (2) six of the seven International Agency for Research on Cancer (IARC) criteria for existence of the alpha-2u- globulin process have been met[4]; and (3) EPA has not found other information or data to suggest that another mechanism is likely to be involved.

Based on this evaluation, the Agency believes that DINP-induced kidney tumors are

[3] The EPA criteria are (1) an increase in number and size of hyaline (protein) droplets in kidney proximal tubule cells of treated male rats; (2) immunohistochemical evidence of alpha-2u-globulin accumulating protein in the hyaline droplets; and (3) histopathological evidence of kidney lesions associated with alpha-2u-globulin nephropathology.

[4] The IARC criteria that EPA believes have been satisfied are (1) lack of genotoxicity activity; (2) male rat specificity for nephropathy and renal tumorigenesis; (3) induction of the characteristic sequence of histopathologicalchanges in shorter-term studies of which protein droplet accumulation is obligatory; (4) identification of the protein accumulating in tubular cells as alpha-2u-globulin; (6) induction of sustained increased cell proliferation in the renalcortex; and (7) similarities in dose-response relationship of the tumor outcome with histopathological endpoints (protein droplets, alpha-2u-globulin accumulation, cell proliferation). The seventh criterion, which has not been met by these data, provides evidence that demonstrates that the chemical of interest (i.e., DINP) binds to alpha-2u-globulin.

associated with a male rat-specific mechanism involving alpha-2u-globulin accumulation in the kidney and that this mechanism is not appropriate for estimating hazard in humans.

iii. Mononuclear Cell Leukemia (MNCL)

The incidence of MNCL was significantly elevated in male and female rats exposed to DINP in the diet when compared to study control animals and the corresponding spontaneous/background incidence in two independent chronic/carcinogenicity rat studies (Lington et al. 1997, Moore 1998a). The key issue in use of these data to assess the hazard of DINP exposure is the relevance of MNCL to human health.

MNCL, also referred to as large granular lymphocyte (LGL) leukemia or T (lymphocyte) leukemia, is a spontaneously occurring neoplasm of the hematopoietic system that is one of the most common tumor types in the Fischer-344 rat strain. MNCL is life threatening in Fischer rats and results in a decreased life span. In contrast, MNCL is rare in other strains of rats and does not occur in mice. Although MNCL is recognized as a common neoplasm in Fischer rats, the MOA for induction of MNCL is not completely understood. In addition, there are differing views on the existence of a close human correlate to MNCL (Caldwell 1999; CHAP 2001).

The increased mortality due to MNCL in DINP-treated rats suggests that DINP is associated with the elevated incidence, progression, and severity of MNCL. The tumor findings may be biologically significant because the time to onset of tumor was decreased and the disease was more severe in treated than in control animals. On the basis of these data, the Agency believes that the increase in severity of MNCL with increasing dose in male rats is indicative of a carcinogenic response to DINP. However, the Agency notes that there are several sources of uncertainty in the interpretation of the experimental data. These include high and variable background rate and possible strain-specificity as well as lack of information on the MOA for induction of MNCL. As a result of this scientific uncertainty, EPA reserves judgement on the human significance of MNCL and whether DINP can reasonably be anticipated to cause cancer in humans.

iv. Summary

DINP has been tested in several carcinogenicity studies in rats and mice. Statistically significant increases in many tumor types were observed, such as increase in hepatocellular

tumors, mononuclear cell leukemia of the spleen, and renal tubular cell carcinomas. In addition, other tumor types considered rare and/or uncommon were noted in DINP-treated animals, including renal transitional cell carcinoma, pancreatic islet cell carcinoma, testicular interstitial (Leydig) cell carcinoma, and uterine adenocarcinoma.

Potential evidence for carcinogenicity of DINP is provided by multiple chronic studies in rats and mice exposed via oral route. Treatment-related increases in tumors were observed at several sites. Non-significant increases in several tumor types considered either rare or uncommon in the tissue and species of origin were also observed in DINP-treated animals (Biodynamics study, 1986; Lington et al. 1997; Moore 1998a and 1998b).

To date, DINP has been classified for its potential carcinogenicity by the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency (CalEPA), but not by any other international agencies. OEHHA has published a document on the evidence on the carcinogenicity of DINP in which members of the Carcinogen Identification Committee (CIC) conclude that DINP has been clearly shown, through scientifically valid testing according to generally accepted principles, to cause cancer and should be listed under Proposition 65 as a carcinogen (OEHHA 2013a). Accordingly, DINP was listed under Proposition 65 at the end of 2013 (OEHHA 2013b). California OEHHA cites evidence from multiple studies in mice and rats to support the Proposition 65 listing of DINP, including identification of:

- liver tumors in female SD rats;
- liver tumors in male and female F344 rats;
- liver tumors in male and female B6C3F1 mice;
- mononuclear cell leukemia (MNCL) in male and female F344 rats;
- renal tubular cell carcinomas, which are rare or uncommon, in male F344 rats;
- renal transitional cell carcinomas, which are rare, in male F344 rats;
- pancreatic islet cell carcinomas, which are rare, in male SD rats and female B6C3F1 mice;
- testicular interstitial (Leydig) cell carcinomas, which are uncommon, in male SD rats; and
- uterine adenocarcinomas, which are rare, in female SD rats

DINP, like most of the phthalates, was negative in the limited number of genotoxicity assay systems and ruled-out as a genotoxic carcinogen; however, that determination leaves

the non-genotoxic mechanisms for consideration for plausible carcinogenic mechanism. DINP has been found to induce *in vitro* cell transformation in only one out of eight studies conducted with Balb/c-3T3 A31 mouse cells. DINP binds to PPAR and activates both rodent and human PPAR alpha and PPAR gamma but not PPAR beta receptors. MINP, the metabolite of DINP, activated both the mouse and human PPAR alpha and PPAR gamma receptors, but the degree of PPAR alpha and PPAR gamma activation was greater for the mouse receptor than for the human receptor for both receptor types in the tested conditions.

DINP has been shown to activate human CAR and PXR and the metabolites of DINP, specifically MiNP, activates hCAR2 isoform, suggesting that DINP and its metabolites have more than one MOA (Laurenzana et al. 2016). DINP has also been shown to promote and induce tumorigenesis in a variety of cell types through AhR-mediated genomic and nongenomic pathways (Wang et al. 2012). DINP induces several changes in rodent liver consistent with PPAR alpha activation. DINP induces some of these liver changes independently of PPAR alpha activation as shown in PPAR alpha-null mice.

TNF-α plays a pivotal role in a number of cell signaling pathways involved in inflammation, cell proliferation, and apoptosis; however, these have not been consistently reported DINP treated mice and rats. TNF-α functional perturbation might contribute to carcinogenesis. In studies conducted in a human promonocyte cell line, DINP reduced phagocytosis in a dose-dependent manner and increased TNF-α levels. DINP is shown to inhibit hepatic GJIC and the inhibition of GJIC has been proposed as a non-genotoxic carcinogenic mechanism, in rodents exposed to DINP for 2 or 4 weeks.

In the read across approach, DINP is structurally similar to di(2-ethylhexyl)phthalate (DEHP) and butyl benzyl phthalate (BBP). All three phthalates are carcinogenic in rodents, are metabolized via similar detoxification pathways, and have similar modes of action (e.g., PPAR alpha is believed to play a role in liver tumorigenesis for all three phthalates). Due to these similarities, DEHP and BBP carcinogenicity data may be informative for a read-across approach to DINP. DEHP has been classified by IARC as a Group 2B (*possibly carcinogenic to humans*) carcinogen (IARC 2013, 2017); by EPA as a Class B2 (*Probable human carcinogen*) carcinogen (IRIS 1988); by the National Toxicology Program (NTP) to be *reasonably anticipated to be a human carcinogen* (NTP 2016); and is listed by CalEPA under Proposition 65 as causing cancer (OEHAA 2003). EPA classified BBP as a Class C "possible human carcinogen" in 1993; however, this classification will be reassessed as part of the

upcoming risk evaluation of BBP under TSCA. In 2000, IARC determined that the evidence of the carcinogenicity of BBP in humans was inadequate, the evidence in experimental animals was limited, and concluded that BBP is *not classifiable as to its carcinogenicity to humans* (Group 3) (IARC 1999). Previous assessments indicate DEHP is a carcinogenic hazard to humans. Based on structural and mechanistic similarities of DEHP to DINP, DINP may also be a carcinogenic hazard to humans.

A list of the studies considered for the chronic exposure in rodents is presented along with a brief summary of results in [ REF _Ref99637485 \h * MERGEFORMAT ].

**Table [ SEQ Table * ARABIC ]. Chronic Toxicity Studies in Rodents**

| Strain Route, Duration, and Reference | Result |
|---|---|
| Fischer 344 rats; 0, 500, 1,500, 6,000, and 12,000 ppm; est. 0, 29, 88, 359, 733 (males); 0, 36, 109, 442, 885 (females); Diet; 2 years<br><br>Recovery study; 0, 12,000 ppm; est. 0, 637 (males); 0, 774 (females); Diet; 78 weeks, followed by 26 weeks recovery<br><br>(Moore 1998a) | Dose-related increase in incidence of MNCL in both sexes from 6,000 ppm (males: 22/65, 23/50, 21/50, 32/65, 30/65 at 0, 29, 88, 359, 733 mg/kg bw/day, respectively; females: 17/65, 16/49, 9/50, 30/65, 29/65 at 0, 36, 109, 442, 885 mg/kg bw/day, respectively).<br><br>Significant increase in hepatocellular carcinoma in males at the highest dose tested (1/65, 0/50, 0/50, 1/65, 12/65 at 0, 29, 88, 359, 733 mg/kg bw/day, respectively) but not in females (1/65, 0/49, 0/50, 1/65, 5/65 at 0, 36, 109, 442, 885 mg/kg bw/day, respectively). Significant increase incidence of carcinoma or adenoma in both sexes at the highest dose (males: 5/65, 3/50, 2/50, 7/65, 18/65 at 0, 29, 88, 359, 733 mg/kg bw/day, respectively; females: 1/65, 1/49, 0/50, 2/65, 8/65 at 0, 36, 109, 442, 885 mg/kg bw/day, respectively).<br><br>LOAEL (non-neoplastic): 358- 442 mg/kg bw/day (increase in absolute and relative liver and kidney weights, increase in serum ALT and AST, and histopathological findings in both organs) (males/females)<br><br>Recovery study: Significant increase in MNCL in both sexes (31/50 and 24/50 in recovery males and females, respectively) and significant increase in renal tubular carcinomas in males (0/65, 4/50 at 0, 637 mg/kg bw/day, respectively). |
| Fischer 344 rats; 0, 0.03, 0.3, 0.6%; est. 0, 15, 152, 307 (males); 0, 18, 184, 375 (females); Diet; 2 years<br><br>(Lington et al. 1997) | Increase in incidence of MNCL at the two highest doses tested in both sexes (males: 33/81, 28/80, 48/80, 51/80 at 0, 15, 152, 307 mg/kg bw/day, respectively; females: 22/81, 20/81, 30/80, 43/80 at 0, 18, 184, 375 mg/kg bw/day, respectively).<br><br>LOAEL (non-neoplastic): 152 mg/kg/day (males) and 184 mg/kg/day (females) (increase in absolute and relative liver and kidney weights, and |

| Table [ SEQ Table * ARABIC ]. Chronic Toxicity Studies in Rodents | |
|---|---|
| **Strain Route, Duration, and Reference** | **Result** |
| | increase in histopathological changes in both organs at the two highest doses) (males/females) |
| Sprague-Dawley rats; 0, 500, 5,000, 10,000 ppm; est. 0, 27, 271, 553 (males); 0, 33, 331, 672 (females); Diet; 2 years<br><br>(Bio/dynamics 1986) | Microscopic evaluation of the liver in all treated groups and other select tissues in the high-dose only suggested the following compound-related changes: (1) hepatocellular carcinomas in mid- and high-dose males and females; (2) minimal to slight focal hepatocellular necrosis in the treated males; (3) testicular interstitial cell hyperplasia in the high-dose males; and (4) renal medullary mineral deposit in the high-dose males. In addition, slightly increased incidences of pancreatic islet cell tumors and parathyroid gland hyperplasia were observed in high-dose males and endometrial hyperplasia was observed in high-dose females.<br><br>The LOAEL for non-cancer systemic effects in males is 500 ppm based on increased incidence of hepatic necrosis; a NOAEL was not established. The LOAEL for non-cancer systemic effects in females is 5,000 ppm based on increased liver weight; the NOAEL is 500 ppm (27 mg/kg/day). The test substance was carcinogenic in females and possibly also in males under the conditions of this study based on the data provided for hepatocellular carcinoma. Findings for testicular interstitial cell tumors were equivocal.<br><br>Study Limitations: Although effects were observed upon histopathological evaluation of the kidneys, testes, and uterus, no histopathological evaluation was conducted on samples from the low- or mid-dose groups, which limits the assessment of dose-dependency and effect levels. |
| B6C3F1 mice; 0, 500, 1,500, 4,000, 8,000 ppm; est. 0, 90, 276, 742, 1,560 (males); 0, 112, 336, 910, 1,888 (females); Diet; 2 years<br><br>Recovery study; 0, 8,000 ppm; est. 0, 1,377 (males); 0, 1,501 (females); Diet; 78 weeks, followed by 26 weeks recovery<br><br>(Moore 1998b) | Significant increase in incidence of hepatocellular carcinoma at the two highest doses in females and at the highest dose in males (males: 10/70, 8/67, 10/66, 17/65, 20/70 at 0, 90, 276, 742, 1560 mg/kg bw/day, respectively; females: 1/70, 2/68, 5/68, 7/67, 19/70 at 0, 112, 336, 910, 1888 mg/kg bw/day, respectively). Significant increase in incidence of total liver neoplasms (carcinomas and adenomas) in females from 336 mg/kg bw/day and in males at the two highest dose (males: 16/70, 13/67, 18/66, 28/65, 31/70 at 0, 90, 276, 742, 1,560 mg/kg bw/day, respectively; females: 3/70, 5/68, 10/68, 11/67, 33/70 at 0, 112, 336, 910, 1888 mg/kg bw/day, respectively).<br><br>LOAEL (non-neoplastic): 276 mg/kg bw/day (male) 336 mg/kg bw/day (female) (increase in absolute liver weights accompanied with histopathological changes in the liver at the highest dose and decreased body weight gain) (females); (increased incidence of liver masses and |

| Table [ SEQ Table * ARABIC ]. Chronic Toxicity Studies in Rodents | |
|---|---|
| **Strain Route, Duration, and Reference** | **Result** |
| | decreased absolute kidney weights) (males)<br><br>Recovery study: Increased incidence of total liver neoplasms in both sexes. Significant increased incidence of carcinoma in females only. |
| Pathology Working Group Review of Data for Spongiosis Hepatis and MNCL (EPL 1999)<br>A Histopathology Peer Review and a Pathology Working Group Review (PWG) were conducted on selected lesions of the liver and spleen observed in Fischer-344 rats in the 2-year bioassays reported by Lington et al. (1997) and Moore (1998a).<br>The PWG review evaluated the significance of spongiosis hepatis, foci of cellular alteration, primary hepatocellular neoplasms inthe liver, and the significance of mononuclear cell leukemia. | The results of the Histopathology Peer Review and PWG generally confirmed the original findings of the study pathologist(s). The PWG concluded that when data from both studies were considered, the NOAEL for DINP is 1,500 ppm (88 mg/kg/day) in male rats, based on the increased incidence of hepatic spongiosis in males at concentrations of 3,000 ppm (152 mg/kg/day) and greater. The PWG identified a NOAEL of 3,000 ppm (184 mg/kg/day) in females, based on increased incidence of MNCL at concentrations of 6,000 ppm (375 mg/kg/day) or higher. |

## VII. Genotoxicity

The genotoxicity of DINP has been assessed in assays of mutagenicity, chromosome aberration, unscheduled DNA synthesis and other relevant endpoints. Although the genotoxicity of DINP metabolites has not been directly investigated, the available data for DINP genotoxicity include results obtained in the presence of metabolic activating systems.

1. Mutagenicity

DINP has been tested for mutagenicity in *Salmonella typhimurium* reverse mutation assays with consistently negative results. The EG&G Mason Research Institute (TSCATS

1980) obtained negative results for mutagenic activity in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of Aroclor-induced rat liver S9 fraction in the plate incorporation assay at DINP (CASRN 28553-12-0) concentrations ranging from 0.1–10 microliter (µl)/plate. In a preliminary test, DINP was not cytotoxic at concentrations up to and including 10 µl/plate. Zeiger et al. (1985) obtained negative results in tester strains TA98, TA100, TA1535, and TA1537 at DINP (CASRN 28553-12-0) concentrations of 0 to 10,000 micrograms µg/plate in the presence and absence of rat or hamster liver S9 fraction. Dimethyl sulfoxide (DMSO) was used as the vehicle. BASF (1986, 1995) obtained negative results in tester strains TA98, TA100, TA1535, TA1537 in the presence and absence of DINP (CASRN 28553-12-0) in DMSO at concentrations ranging from 20–5000 µg/plate. McKee et al. (2000) obtained negative results for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested at DINP (CASRN 68515-48-0) concentrations of 0.5–5000 µg/plate.

The mutagenic potential of DINP in mammalian cells has been evaluated *in vitro* using mouse L5178Y lymphoma cells. DINP did not induce forward mutations at the thymidine kinase (TK+/-) locus of L5178Y cells when tested at concentrations ranging from 7.5–100 µl/mL in the presence and absence of metabolic action by Aroclor-induced rat liver S9 fraction (TSCATS 1981b). The investigators reported that DINP was immiscible with culture media under the conditions of this test. Barber et al. (2000) obtained negative results for forward mutations at the TK locus in cultured L5178Y cells in assays conducted with and without activation by S9 rat liver fraction. The tested concentrations ranged from 1.5–8.0 µl/mL for assays in the absence of metabolic activation and from 0.05 to 0.6 µl/mL for assays in the presence of S9 fraction.

2. Chromosomal Aberrations

In an *in vivo* cytogenetic assay, Microbiological Associates (1981; TSCATS Doc # 878210231) determined the frequency of chromosomal aberrations in femoral bone marrow cells of male Fischer-344 rats (5/dose) given DINP (CASRN 28553-12-0) by oral gavage once daily for 5 days consecutively. Animals concurrently dosed with olive oil or triethylenemelamine served as negative and positive controls, respectively. Administration of 0.5. 1.7, or 5.0 mg/kg/day in corn oil did not induce statistically significant clastogenic effects. DINP did not affect the mitotic index in bone marrow cells at any tested dose and was

non-toxic in a preliminary range finding assay at treatment levels up to and including 50 mL/kg/day. These data suggest that an adequately high-dose of DINP may not have been tested in that assay. In addition, the cytogenetic analysis was performed on only 50 cells/animal and the mitotic index was calculated by observation of only 100 cells. These sample sizes are less than recommended in standard protocols and may not have been sufficient to detect low level effects.

DINP was tested for clastogenic activity in cultured Chinese hamster ovary (CHO) cells at concentrations of 40, 80, and 160 µg/ml (in acetone) in the presence or absence of an exogenous metabolic system from Aroclor-induced Sprague Dawley rat livers (McKee et al. 2000). The study consisted of two phases: (1) an initial 20-hour harvesting chromosomal aberration assay (with a 3-hour exposure with and without metabolic activation), and (2) a repeat assay with both 20- and 44-hour cell harvest times (with a 3-hour exposure with metabolic activation and 20-hour exposure without metabolic activation). Frequencies of chromosome aberrations were similar in DINP-treated and control groups at all concentrations. The positive controls, dimethylbenz[a]anthracene and nitrosoguanidine, both produced statistically significant increases in the percent of aberrant cells. Under the conditions of this study, DINP did not induce chromosomal aberrations in CHO cells.

Negative results were obtained for induction of micronuclei in male CD-1 rats (5/dose) given DINP by oral gavage at concentrations of 500, 1000, or 2000 mg/kg for 2 days. An initial range finding study indicated that there was no difference between male and female response to DINP (McKee et al. 2000).

The effect of DINP on unscheduled DNA synthesis was examined in the rat hepatocyte primary culture/DNA repair assay (Litton Bionetics 1981; TSCATS Doc. # 878210229). Based on preliminary cytotoxicity tests, DINP was tested using solutions of 0, 0.625, 1.25, 2.5, 5.0, and 10.0 µl/ml in DMSO, which were further diluted to 1:100 with culture medium. Survival ranged from 93–100%. None of the tested concentrations produced statistically significant increases in unscheduled DNA synthesis when compared to the solvent control.

3. Cell Transformation Assays

The cell transformation potential of DINP has been evaluated in seven studies (ECJRC, 2003). DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells at concentrations of 125–3,750 nL/mL (Litton Bionetics 1985), 2.5–254.5 µg/mL (Litton

Bionetics 1981a), 0.2–3,260 µg/mL (Litton Bionetics 1981b), or 0.125–3.750 µL/mL (Barber et al. 2000) in the absence of metabolic activation. Slight increases in the number of transformed foci were observed in Balb/c-3T3 A31 cells treated with 0.03–1 µL/mL (Microbiological Associates 1982) without metabolic activation or with 0.1 to 1 µL/mL in the presence (Microbiological Associates 1981a) or absence (Microbiological Associates 1981b) of metabolic activation. However, the responses were not dose-related or statistically significant. Positive results were observed in one assay conducted at concentrations of 0.03–1 µL/mL (Microbiological Associates 1981c) in the absence of metabolic activation.

Fushiwaki et al. (2003) obtained negative results for tumor-promoting potential in Balb/c- 3T3 mouse cells co-cultured with transformed cloned cells (strain 4-1-1) and treated with DINP at concentrations of up to 5.0 µg/mL.

4. Conclusions on Genotoxicity

In the available genotoxicity studies for DINP, negative results were observed in bacterial mutation assays, with and without metabolic activation (EG and G Mason Research Institute 1980; Zeiger 1985; BASF 1986; BASF 1995; Exxon Biomedical Sciences 1996d; Mckee 2000). Negative results were also observed in *in vitro* mouse lymphoma assays and CHO cell chromosomal aberration assays, with and without metabolic activation (EG and G Mason Research Institute 1981; Litton Bionetics 1985a; BASF 1986; Hazleton 1986; Exxon Biomedical Sciences 1996e; Mckee 2000). In addition, DINP did not induce unscheduled DNA repair in primary rat hepatocytes (Litton Bionetics 1981a). DINP also tested negative in an *in vivo* cytogenetics test with bone marrow of rats exposed orally for 5 days (Microbiological associates 1981d) and in an *in vivo* mouse micronucleus assay in which CD-1 mice were given up to 2000 mg DINP/kg-bw per day by oral gavage for 2 days (McKee et al. 2000).

In several *in vitro* transformation studies of Balb/c 3T3 cells, DINP was shown to be positive (without metabolic activation) in one of seven studies (Microbiological Associates 1981a,b,c; Litton Bionetics 1981b,c; Microbiological Associates 1982; Litton Bionetics 1985b). The weight of evidence from these studies supports a conclusion that DINP is not genotoxic.

## VIII. Reproductive and Developmental Toxicity

a) Reproductive Toxicity

1. Oral One- and Two-Generation Reproductive Toxicity Studies in Rats (Waterman et al. 2000)

The potential reproductive toxicity of DINP has been assessed in one- and two-generation studies conducted in Sprague-Dawley rats. The studies were conducted according to recommendations in EPA guidelines and were GLP-compliant. The one-generation study was conducted as a pilot study for the two-generation study in order to identify appropriate doses. In the one-generation study, groups of 30 male and female animals were administered DINP at 0, 0.5, 1.0, or 1.5% w/w in the diet for 10 weeks prior to mating and throughout the mating period. The females were subsequently exposed throughout gestation and lactation until postnatal day (PND) 21. Estimated doses for males during the premating period were 301–591, 622–1,157, and 966–1,676 mg/kg/day in the low-, mid- and high-dose groups, respectively (as reported in NTP-CERHR, 2003). Estimated doses for the low-, mid- and high-dose females were 363–624, 734–1,169, and 1,114–1,694 mg/kg/day during premating; 377–404, 741–796, and 1,087–1,186 mg/kg/day during gestation; and 490–923, 1,034–1,731, and 1,274–2,246 mg/kg/day during lactation (as reported in NTP-CERHR, 2003). Evaluated parameters included parental and offspring survival, clinical signs, body weights and body weight gain, and male and female fertility. After sacrifice, major organs of parental animals were grossly examined and weighed.

In the one-generation study, body weight gain was significantly reduced at the 1 and 1.5% levels of DINP in both sexes during the premating phase and in females during gestation and lactation. Absolute liver and kidney weights in both sexes were significantly elevated at all doses, except in females at the 1.5% level. Absolute testes weights were significantly increased, and ovarian weights were significantly decreased (30%) at the 1.5% level. There were no significant effects on indices of mating or fertility (e.g., litter size) and a reproductive NOAEL of 1.5% (~1,000 mg/kg/day) was identified. Live birth index and survival of offspring during lactation were significantly reduced in the 1.5% treatment group. Body weights at PND 0, 14 and 21 were significantly reduced in offspring from all treatment groups at a LOAEL of 377–923 mg/kg/day.

For the two-generation study, four groups of male and female Sprague-Dawley rats (30/sex/dose; designated P1) were fed DINP at dietary concentrations of 0.0, 0.2, 0.4, or 0.8% for 10 weeks before mating and for an additional 7 weeks, through mating, gestation, and

lactation. Dietary DINP concentrations of 0, 0.2, 0.4 and 0.8% corresponded to doses of 0, 165, 331, and 665 mg/kg/day for males during premating; 0, 182, 356, and 696 mg/kg/day for females during premating; 0, 143–146, 287–288, and 555–560 mg/kg/day for females during gestation; and 0, 254–285, 539–553, and 1,026–1,129 mg/kg/day for females during lactation (as reported in NTP-CERHR, 2003). After 10 weeks of dietary DINP exposure, randomly selected male and female rats in the P1 generation were paired 1:1 within dose groups to mate and produce offspring, which were designated as P2(F1). On PND 4, the offspring were culled to yield four pups per sex per litter after being counted, weighed, and examined. As each litter reached PND 21, male and female pups were randomly selected as parents for the F2 generation. When possible, each litter was represented with at least one pup. The remaining F1 pups were sacrificed following weaning and examined. The P2 (F1) rats (30/sex/dose) continued on the DINP diet from PND 21. Estimated doses for the F1 rats were 0, 189, 379, and 779 mg/kg/day for males during premating; 0, 197, 397, and 802 mg/kg/day for females during premating; 0, 143, 288, and 560 mg/kg/day for females during gestation; and 0, 285, 553, and 1,129 mg/kg/day for females during lactation (as reported in NTP-CERHR, 2003). Following mating, P2 females were allowed to litter and raise young until weaning, at which time the F2 litters and adults were sacrificed and examined.

Mortality, clinical signs, body weights, and feed consumption were monitored in adult and weanling animals. Litters from the P1 and P2 generations were counted, sexed, weighed and examined externally on PND 0, 1, 4, 7, 14, and 21. Complete postmortem examinations were conducted on all animals in the study. Organ weights were collected for liver, kidneys, brain, and reproductive organs in animals that survived to study termination. Gross lesions and reproductive and accessory organs from control and high-dose animals were examined microscopically.

There were no treatment-related effects on parental survival at any dose in the two-generation study. No dose-related clinical signs of toxicity were observed. The mean body weights of P1 females in the 0.8% group were statistically significantly reduced at PND 14 and 21 relative to the control. Absolute liver and kidney weights of P1 males and females were increased over controls at all DINP treatment levels. There were no significant differences in male mating, male fertility, female fertility, female fecundity, gestational indices, or length of gestation. Sex ratios and survival during lactation were unaffected in the F1 generation. The mean litter sizes of the treated groups were significantly greater (18–21%)

than the control group. PND 0 weights were significantly reduced in male offspring from the 0.8% group. Significant reductions in the body weight gain of offspring were also observed in the 0.4 and 0.8% groups on PND 7, 14, and 21.

Exposure to DINP did not result in any unscheduled deaths or treatment-related signs in P2 males or females. At the time of selection (approximately 6 weeks after weaning), dose-related decrements in body weight gain were noted in males and females in the 0.4 and 0.8% groups. Females in the 0.8% group had consistently lower body weight gain than the controls during the premating (significant for first 2 weeks), gestation (not significant), and lactational (significant for PND 4–21) phases. There were no significant effects on standard reproductive indices. PND 0 offspring weight gain for the F2 generation did not differ significantly from controls when litter size differences were considered. Offspring weights were significantly reduced in the 0.4 and 0.8% groups during lactation when compared to the controls. There were no effects observed on the reproductive organ weights or histopathological examinations. No gross effects were noted in P2 animals or their offspring at necropsy.

Microscopic examination of the livers of parental animals in both generations revealed minimal to moderately increased cytoplasmic eosinophilia in males and females from all DINP treatment groups in both generations. The study authors noted that this lesion is often associated with peroxisome proliferation. Microscopic examination of the kidneys revealed an increased incidence of dilatation of the renal pelvis in P2 males at the 0.4 and 0.8% groups.

The NOAEL for reproductive effects in this study appears to be 0.8% (665–779 mg/kg/day for males and 696–802 mg/kg/day for females), the highest dietary concentration tested. The significantly reduced weight gain observed in F1 pups at PND 21 suggests a developmental LOAEL of 0.2% (143–285 mg/kg/day for gestation/lactation), the lowest dietary concentration tested.

Landmarks of sexual maturation (i.e., preputial separation, anogenital distance, nipple retention, biochemical and structure of the developing reproductive system) were not examined by Waterman et al. (2000). However, the effect of DINP on the sexual differentiation of rats has been tested elsewhere by Gray et al. (2000; see study summary under Developmental Toxicity) in a perinatal developmental study.

b) Developmental Toxicity

Evidence for developmental toxicity of DINP has been obtained in two prenatal oral studies (Hellwig et al. 1997; Waterman et al. 1999), a two-generation study (Waterman et al. 2000), and a perinatal study of male sexual maturation (Gray et al. 2000).

1. Prenatal Oral Developmental Toxicity Study in Rats (Waterman et al. 1999)

The developmental toxicity of DINP (DINP-1; CASRN 068515-48-0) has been investigated in a prenatal developmental toxicity study conducted in Sprague-Dawley rats (Waterman et al. 1999). DINP was administered by gavage in corn oil to mated rats (25/group) at doses of 0, 100, 500, or 1,000 mg/kg/day on GDs 6–15. Dams were monitored for viability, clinical signs, body weight, and food consumption. Caesarean sections were performed on GD 21 and the fetuses removed for evaluation of skeletal malformations and variations and soft tissue anomalies. Implantation sites, resorptions, and the number of live and dead fetuses were recorded.

All females survived to scheduled study termination on GD 21. Maternal weight gain was significantly reduced at 1000 mg/kg/day at the initiation of treatment (GD 6–9 and for the overall treatment period (GD 6–15). No significant changes in maternal body weight were observed for the entire gestation period (GD 0–21). Food consumption at 1,000 mg/kg/day was significantly reduced for GD 9–12, with a non-significant decrease noted for the overall (GD 6–15) treatment interval. On the basis of these data, the study authors identified NOAEL and LOAEL values of 500 and 1,000 mg/kg/day, respectively, for maternal toxicity. Mean numbers of corpora lutea, total implantation sites, post-implantation loss, and viable fetuses were comparable to the control values. Fetal body weights and sex ratios were not adversely affected by exposure to DINP.

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Fetal Variations in Offspring of Sprague-Dawley Rats Treated with DINP on GD 6-15 (Waterman et al. 1999)**

| Effect | Unit of Analysis | Data Source | Dose (mg/kg/day) (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | Control | 100 | 500 | 1,000 |
| Skeletal variations | | | | | | |
| Overall skeletal variations | % Fetuses | (a) | 16.8 | 15 | 28.4* | 43.7* |
| | % Fetuses in litter | (b) | 16.4 | 15 | 28.3* | 43.4* |

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Fetal Variations in Offspring of Sprague-Dawley Rats Treated with DINP on GD 6-15 (Waterman et al. 1999)**

| Effect | Unit of Analysis | Data Source | Dose (mg/kg/day) (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | Control | 100 | 500 | 1,000 |
| | % Litters | (a) | 62.5 | 64 | 91.7* | 87 |
| Rudimentary lumbar ribs | % Fetuses | (a) | 3.7 | 5.4 | 18.6* | 34.5* |
| | % Fetuses in litter | (b) | 3.5 | 4.7 | 18.1* | 34.2* |
| | % Litters | (a) | 25 | 20.2 | 54.2 | 78.3* |
| Supernumerary cervical ribs | % Fetuses | (a) | 1.6 | 1.6 | 1.0 | 5.7* |
| | % Fetuses in litter | (b) | 1.6 | 1.5 | 1.0 | 5.5* |
| | % Litters | (a) | 12.5 | 12 | 8.3 | 30.4 |
| Visceral variations | | | | | | |
| Overall visceral variations | % Fetuses | (a) | 0.5 | 3.7* | 4* | 5.1* |
| | % Fetuses in litter | (b) | 0.5 | 3.3 | 3.7 | 5.8* |
| | % Litters | (a) | 4.2 | 12 | 16.7 | 30.4* |
| Dilated renal pelves | % Fetuses | (a,b,c) | 0 | 3.7 | 4 | 4.5* |
| | % Litters | (a,c) | 0 | 12 | 16.7 | 26.1* |

Source: Table 3 in Waterman et al. (1999) and Tables 5 and 6 in the NTP-CERHR Panel Report (NTP-CERHR 2000)

* = Significantly different from the control at $p \leq 0.05$

(a) Data are from the original analysis performed by Waterman et al. (1999).

(b) Re-analysis of original data by Waterman et al. (1999) using the GEE statistical method; unpublished results reported in (NTP-CERHR 2000).

(c) An alternative statistical analysis that considers litter effects was used; the GEE method could not be applied because the incidence in the control group was zero (NTP-CERHR 2000).

a) Skeletal Variations

Exposure to DINP produced skeletal variations but not malformations. The available data for skeletal variations ([ REF _Ref99637552 \h * MERGEFORMAT ]) include the original analysis of the experimental results as published by Waterman et al. (1999) and a reanalysis of that data performed and reported in the NTP Center for Evaluation of Risks to Human Reproduction (NTP-CERHR) Expert Panel Report (NTP-CERHR 2000; data from this report are also summarized in Kavlock et al. 2002). In the original analysis, fetal results were presented on the basis of affected litters or fetuses. Skeletal variations were observed at 500 mg/kg/day and 1,000 mg/kg/day levels when examined for litters or fetuses. The most prominent skeletal variant was presence of rudimentary lumbar ribs. A dose-related increase

in litters with rudimentary lumbar ribs (25.0, 20.2, 54.2 and 78.3%) was observed and reached statistical significance at the high-dose in the original analysis reported by the study authors. A dose-related increase in the percent of fetuses with rudimentary lumbar ribs (3.7, 5.4, 18.6, and 34.5%) for the 0, 100, 500 and 1,000 mg/kg/day, respectively, was observed and reached statistical significance at 500 and 1,000 mg/kg/day. The fetal incidence of supernumerary ribs on the seventh cervical vertebrae (1.6, 1.6, 1.0, and 5.7%) was significantly increased at 1,000 mg/kg/day, but the litter incidence for supernumerary cervical ribs (12.5, 12.0, 8.3, and 30.4%) did not reach statistical significance at any dose.

In addition to the statistical analysis reported in the original publication, the original study authors conducted a reanalysis of the fetal incidence data using improved and more current statistical procedures. The results of the reanalysis, reported in NTP-CERHR (2000), indicate that the mean percentages of fetuses per litter with rudimentary lumbar ribs or skeletal variations were significantly greater in the 500 and 1,000 mg/kg/day groups than in the control group percentage ([ REF _Ref99637552 \h * MERGEFORMAT ]). These results indicate that the LOAEL for developmental toxicity in the Waterman et al. (1999) study is 500 mg/kg/day if based on fetal incidences of total skeletal variations or rudimentary lumbar ribs. Thus, developmental effects occurred at doses of DINP that did not cause maternal toxicity. This interpretation is consistent with the conclusions reached on DINP by the NTP-CERHR Expert Panel (2000).

In reviewing the data from this study, EPA notes that supernumerary ribs in the cervical region are uncommon and are a concern for that reason. The presence of extra cervical ribs may indicate a disruption of gene expression, and there is evidence that cervical ribs may interfere with normal nerve function and blood flow (NTP-CERHR 2000). Therefore, although the biological significance of a statistically significant increase in rib variations is uncertain, the Agency believes that the dose-related response observed in the Waterman et al. (1999) study may represent growth alterations that are indicative of DINP's potential to disrupt normal developmental patterns and produce a developmental hazard.

b) Kidney Effects

Waterman et al. (1999) showed that there was a positive, dose-related trend in the percentage of fetuses and litters with visceral variations, predominately dilated renal pelvises. The incidence data for dilated renal pelvises are shown in [ REF _Ref99637552 \h *

MERGEFORMAT ]. The litter incidences for dilated renal pelvises were 0, 12.0, 16.7, and 26.1% for the control, 100, 500, and 1,000 mg/kg/day groups, respectively, and reached statistical significance at 1,000 mg/kg/day. Based on the original analysis presented by the study authors, the percentage of fetuses with dilated renal pelvises was significantly increased at all treatment levels (0, 3.7, 4.0, and 4.5% at 0, 100, 500, and 1,000 mg/kg/day, respectively).

The interpretation of study results by Waterman et al. (1999) included identification of maternal and developmental LOAEL values of 1,000 mg/kg/day and the corresponding NOAEL of 500 mg/kg/day, with a conclusion that DINP "is not teratogenic or a selective developmental toxicant." The expert panel agreed with the authors selection of a maternal NOAEL; however, the panel was concerned that the fetal incidence data for dilated renal pelvises indicated that developmental toxicity could be present at maternal doses of 500 or 100 mg/kg/day. At the suggestion of the panel, the study sponsor reanalyzed the fetal incidence data using improved and more current statistical approaches. The results of the statistical reanalysis are shown in [ REF _Ref99637552 \h * MERGEFORMAT ]. Using the alternative approach, the percentage of fetuses with dilated renal pelvises (0, 3.3, 3.7, and 5.3% at 0, 100, 500, and 1,000 mg/kg/day, respectively) was statistically significant only at the high dose of 1,000 mg/kg/day. The results of the analysis diminished the Panel's concerns that developmental effects based on dilated renal pelvises might extend to doses below 1,000 mg/kg/day. The LOAEL of 500 mg/kg/day in this study was therefore based on incidence data for skeletal variations.

There is some difference of opinion on the long-term significance of dilated renal pelvises in the offspring of pregnant animals exposed to chemicals during gestation. Although some investigators have provided data suggesting that the dilated kidney pelvises represent a transient developmental delay (Woo and Hoar 1972), others have shown that under some circumstances it can persist well into postnatal life and that physiological function is compromised in the affected individuals (Kavlock et al. 1987, 1988). The findings of Kavlock et al. (1987 and 1988). suggest the need for evaluation of potential functional deficits for chemicals that cause dilated renal pelvises, including DINP. Lacking this information, it is important to note that an independent panel of experts in reproductive and developmental toxicology regarded the occurrence of dilated renal pelvises as adverse and considered this response among others as a possible basis for establishment of NOAEL and LOAEL values

for developmental toxicity (NTP-CERHR 2000).

2. Prenatal Oral Developmental Toxicity Study in Rats (Hellwig et al. 1997)

Additional data on the developmental toxicity of DINP are available from a study conducted by Hellwig et al. (1997) who evaluated the potential for three different base stocks containing DINP to cause developmental toxicity in rats. The three DINP formulations were manufactured using different alcohol starting materials, which were described as primary dimethyl heptanol (formulation designated DINP-1; CASRN 68515-48-0), alkyl-substituted octanol or heptanol (DINP-2; CASRN 28553-12-0), and alkyl-substituted hexanol (DINP-3; also listed as CASRN 28553-12-0 but resulting from a different line). DINP-1, -2 and -3 were administered by gavage to 10 Wistar rats/dose at doses of 0, 40, 200, or 1,000 mg/kg/day on GDs 6–15. The dams were sacrificed on GD 20 and implantation sites were examined. The fetuses were weighed and examined for external variations. Half of the fetuses were examined for skeletal malformations and half were examined for visceral variations.

For DINP-1, indications of maternal toxicity at the high-dose were reduced food consumption, increased relative liver weight, and significantly increased relative kidney weight. One dam given 1,000 mg DINP-1/kg-day had vaginal hemorrhage on GD 14. No treatment-related effects were noted on the number of fetuses per dam.

For DINP-2, no statistically significant signs of maternal toxicity were observed at the high-dose of 1,000 mg/kg/day. Small increases in relative liver (5%) and kidney (6%) weight occurred at 1,000 mg/kg/day but did not reach statistical significance. One dam given 1,000 mg DINP-2/kg/day had vaginal hemorrhage on GD 14 and 15. A statistically significant decrease in live fetuses/dam and significant increase in relative kidney weight in dams were observed at the low dose of 40 mg/kg/day, but these effects were not evident at higher doses.

For DINP-3, maternal toxicity was evident at 1,000 mg/kg/day as significantly reduced food consumption during several days of the treatment period, reduced mean body weight gain from GDs 6-15, significantly reduced mean body weight at study termination and significantly increased relative liver weight. No treatment-related effects occurred in the dams receiving doses of 200 or 400 mg/kg/day.

a) Skeletal Variations

Developmental toxicity for DINP-1 was evident at the highest dose as a statistically

significant increase in percent fetuses/litter with skeletal variations (35.3, 41.5, 29.5, and 58.4% in the 0, 40, 200, and 1,000 mg/kg/day groups, respectively) ([ REF _Ref99637663 \h * MERGEFORMAT ]). The variations included rudimentary cervical and accessory 14th rib(s), skeletal variations that showed an apparent dose-related trend for incidence. Shortened 13th ribs were noted in fetuses in the control and 40 mg/kg/day groups, but not at higher doses. No significant skeletal variations were evident at the 40 or 200 mg/kg/day doses, and no fetal weight changes were noted at any dose.

For DINP-2, the study authors reported that developmental toxicity was limited to an increased fetal incidence of accessory 14th lumbar ribs at the high-dose (0% of fetuses in 0% of control litters vs. 7.1% of fetuses in 50% of treated litters).

For DINP-3, developmental toxicity was indicated by a statistically significant increase in percent fetuses/litter with variations at the highest dose (35.3, 29.4, 39.5, and 60.7% in the 0, 40, 200, and 1,000 mg/kg/day groups, respectively). Specific classes of variations observed in the high-dose group included skeletal retardations (unossified or incompletely ossified sternebrae), soft tissue variations (hydroureter), and skeletal variations (rudimentary cervical and/or accessory 14th ribs). In addition, some soft tissue malformations (predominately affecting the urogenital tract) and skeletal malformations (shortened and bent humerus and femur in a single fetus) observed at 1,000 mg/kg/day were considered to be treatment related.

**Table [ SEQ Table * ARABIC ]. Incidence of Selected Fetal Developmental Variations (Hellwig et al. 1997)**

| Test Stock | Type of Variation | Unit of Analysis | Dose (mg/kg/day) | | | |
|---|---|---|---|---|---|---|
| | | | Control | 40 | 200 | 1,000 |
| DINP-1 | Variations | % Fetuses/litter | 35.3 | 41.5 | 29.5 | 58.4* |
| | | % Litters | 100 | 100 | 100 | 100 |
| | Skeletal Variation: Accessory 14th Rib | % Fetuses | 0 | 0 | 1.8 | 28.2 |
| | | % Litters | 0 | 0 | 25.0 | 100 |
| | Skeletal Variation: Rudimentary Cervical Ribs | % Fetuses | 0 | 1.7 | 0.9 | 8.4 |
| | | % Litters | 0 | 11.1 | 12.5 | 50.0 |
| | Dilated Renal Pelvis | % Fetuses | 8.9 | 9.5 | 7.2 | 16.7 |
| | | % Litters | 77.8 | 44.4 | 50 | 90 |
| DINP-2 | Variations | % Fetuses/litter | 35.3 | 37.5 | 40.3 | 36.6 |
| | | % Litters | 100 | 89 | 100 | 100 |

| Table [ SEQ Table * ARABIC ]. Incidence of Selected Fetal Developmental Variations (Hellwig et al. 1997) | | | | | | |
|---|---|---|---|---|---|---|
| **Test Stock** | **Type of Variation** | **Unit of Analysis** | **Dose (mg/kg/day)** | | | |
| | | | **Control** | **40** | **200** | **1,000** |
| | Skeletal Variation: Accessory 14th Rib | % Fetuses | 0 | 0.9 | 3.0 | 7.1 |
| | | % Litters | 0 | 11.1 | 20.0 | 50.0 |
| | Skeletal Variation: Rudimentary CervicalRibs | % Fetuses | 0 | 0 | 0.7 | 2.8 |
| | | % Litters | 0 | 0 | 10 | 40 |
| | Dilated Renal Pelvis | % Fetuses | 8.9 | 8.6 | 16.3 | 10.6 |
| | | % Litters | 77.8 | 66.7 | 70.0 | 80.0 |
| DINP-3 | Variations | % Fetuses/litter | 35.3 | 29.4 | 39.5 | 60.7* |
| | | % Litters | 100 | 100 | 100 | 100 |
| | Skeletal Variation: Accessory 14th Rib | % Fetuses | 0 | 0 | 6.7 | 28.3 |
| | | % Litters | 0 | 0 | 55.6 | 88.9 |
| | Skeletal Variation: Rudimentary CervicalRibs | % Fetuses | 0 | 0 | 1.5 | 10.0 |
| | | % Litters | 0 | 0 | 11.1 | 77.8 |
| | Dilated Renal Pelvis | % Fetuses | 8.9 | 10.9 | 9.6 | 16.7 |
| | | % Litters | 77.8 | 80.0 | 100 | 100 |
| Source: Data compiled from Tables 9 through 14 in Hellwig et al. (1997)<br>*Significantly different from the control at p#0.01; statistical analysis performed by Hellwig et al. (1997) | | | | | | |

b) Kidney Effects

The incidence of dilated renal pelvis was slightly elevated at 1000 mg/kg/day but did not reach statistical significance ([ REF _Ref99637663 \h * MERGEFORMAT ]). The incidences for each DINP stock at the high-dose were 16.7% of fetuses in 90% of DINP-1 treated litters, 10.6% of fetuses in 80% of DINP-2 treated litters, and 16.7% of fetuses in 100% of DINP-3 treated litters—versus 8.9% of fetuses in 78% of control litters (Hellwig et al. 1997). The incidence for this variation was not statistically elevated in any dose group but is considered biologically significant. This variation was observed at a minimal maternally toxic dose for DINP-1 and DINP-3 (as indicated by reduced mean body weight gain and food consumption as well as increased liver and kidney weights). Although the Agency notes that only a mild increase in dilated renal pelvises was noted in rat fetuses exposed to any of the three DINP mixtures, there were additional, more severe renal malformations

(e.g., hydroureter, agenesis or absence of kidney) noted at 1,000 mg/kg/day with DINP-3. The study authors considered these adverse renal malformations to be treatment related. The renal effects reported by Hellwig et al. (1997) support observations of renal toxicity in pups exposed to DINP *in utero* in other reproductive (Waterman et al. 2000) and developmental studies (Waterman et al. 1999).

3. Disposition of Diisononyl Phthalate and Its Effects on Sexual Development of the Male Fetus Following Repeated Dosing in Pregnant Rats (Clewell et al. 2011a)

In a study of male sexual development, timed pregnant Crl:CD Sprague-Dawley rats were administered the test substance in corn oil via oral gavage at target doses of 0 (vehicle), 50, 250, or 750 mg/kg-bw/day (corresponding to mean analytical doses of 0, 47, 242, or 760 mg/kg-bw/day) from gestation days (GDs) 12–19. Groups of dams were sacrificed 2 or 24 hours after the final dose for evaluation of toxicity endpoints. Treated groups consisted of 8 animals/dose group/time point. Each dose group had a separate set of concurrent controls (9 controls/dose group/time point, or a total of 27 control animals per time point). Terminal body weight and body weight gains in treated dams were comparable to controls. Mean absolute and relative maternal liver weights were increased by 12–17% at greater than 242 mg/kg-bw/day. There was no effect on fetal weight or anogenital distance (AGD). Fetal testis testosterone levels were significantly decreased by 50 and 65% at 242 and 760 mg/kg-bw/day, respectively, in fetuses from dams sacrificed 2 hours post-dosing, but did not differ significantly from controls in those sacrificed 24 hours post-dosing. In fetal males sacrificed 24-hours post-dosing, histopathological examination of the testes showed significant increases in multinucleated gonocytes (MNGs) at 242 and 760 mg/kg bw-day and large Leydig cell (LC) aggregates at 760 mg/kg-bw/day. The maternal NOAEL and LOAEL were determined to be 47 and 242 mg/kg-bw/day based on increased liver weights in dams. The developmental NOAEL and LOAEL were determined to be 47 and 242 mg/kg-bw/day based on induction of MNGs and reduced testosterone in fetal testes.

4. Effects after Dietary Administration of DINP in Gestation and Lactation on Male Rat Sexual Development (Clewell 2011b)

In a prenatal developmental toxicity study, timed pregnant female Sprague-Dawley rats (20/group, 24 controls) were administered the test substance in the diet at target concentrations of 0 (base diet), 760, 3,800, or 11,400 ppm (target doses of 0, 50, 250, or 750

mg/kg-bw/day, respectively) from GD 12 through PND 14. Dams were monitored for body weight and food consumption during the study and sacrificed on PND 21. Offspring were evaluated on PND 2, PND 14, or close to adulthood on PND 49/50. Dams exhibited reduced body weight, body weight gain, and food consumption during gestation and lactation at 11,400 ppm. Results in pups were reported only for the males. Male pup body weights were reduced at 11,400 ppm on PND 2 and at both 3,800 ppm and 11,400 ppm on PND 14. AGD was not reduced on PND 2, but was reduced in the 11,400 ppm pups on PND 14. Histopathological findings in the testes on PND 2 included increased MNGs at greater than 3,800 ppm and increased large Leydig cell (LC) aggregates at 11,400 ppm. There were no effects on testes testosterone levels (PND 2) or nipple retention (PND 14) in the pups. In male offspring close to adulthood at PND 49/50, there were no effects on body weight; absolute or relative liver, kidney, or reproductive organ weights; preputial separation; nipple/areola retention; genital malformations; histopathological effects in testes; or testes levels of testosterone. The LOAEL for maternal effects is 11,400 ppm (~750 mg/kg-bw/day) based on reduced body weight, body weight gain, and food consumption during gestation and lactation; the NOAEL is 3,800 ppm (~250 mg/kg-bw/day). The developmental LOAEL is 3,800 ppm (~250 mg/kg-bw/day) for effects seen in male pups, including reduced pup weight and increased MNGs at greater than 3,800 ppm and decreased AGD and increased LC aggregation at 11,400 ppm. The developmental NOAEL is 760 ppm (~50 mg/kg-bw/day).

5. Two-Generation Oral Reproductive Toxicity Study in Rats (Waterman et al. 2000).

DINP caused developmental toxicity in a two-generation rat reproductive study as indicated by significantly reduced mean pup body weight gain in male and female offspring of both generations (F1 and F2) at all doses tested (Waterman et al. 2000). Sprague-Dawley rats were fed DINP at concentrations of 0, 0.2, 0.4 and 0.8% during mating (0, 182–197, 356–397, and 696–802 mg/kg/day); gestation (0, 143–146, 287–288, and 555–560 mg/kg/day); and lactation (0, 254–285, 539–553, and 1,026–1,129 mg/kg/day). Weight gain for F1 pups was significantly reduced for males of the 0.8% group on PND 0, in male and female pups of the 0.4% and 0.8% groups on PND 7 and 14, and in all dose groups on PND 21. In the F2 generation, mean female body weights were significantly reduced in the 0.4 and 0.8% groups on PND 4, 7, 14, and 21 and in the 0.2% group at PND 7. F2 mean male pup weights were significantly reduced at 0.4 and 0.8% at PND 7, 14, and 21. The weight

gain inhibition at PND 21 in F1 pups and PND 7 in F2 pups exposed at the 0.2% level suggests a developmental LOAEL of 143–285 mg/kg/day for the gestation and lactation phases.

6. Perinatal Oral Exposure Study of Male Sexual Differentiation (Gray et al. 2000)

Gray et al. (2000) examined sexual differentiation of neonatal and infant male rats exposed perinatally to DINP (CASRN 68515-48-0). Pregnant Sprague-Dawley rats received daily oral gavage doses of 0 or 750 mg/kg/day (19 or 14 dams, respectively) in corn oil from GD 14 though PND 3. Maternal survival, clinical signs, and body weight were monitored during the gestation period. Body weight and AGD were measured in offspring on PND 2, and one male per litter was sacrificed for measurement of testes weight and histology. At 9–10 days of age, the inguinal region of each pup was examined for hemorrhagic testes. At 13 days of age, the pups were examined for the presence of areolas/nipples. The pups were weaned at PND 28 and examined daily thereafter for the onset of puberty, as detected by preputial separation. The test animals were sacrificed at between 3 and 7 months of age. At the time of sacrifice, blood was collected for measurement of testosterone levels and the males were necropsied. The ventral surface of each animal was examined for abnormalities and an internal examination was also conducted to identify abnormalities of the reproductive system. Reproductive organs were removed and weighed.

Treatment with DINP did not cause maternal death or overt maternal toxicity. Maternal weight gain to GD 21 was significantly reduced (approximately 14%), although maternal weight gain to PND 3 did not differ from the control. In male offspring, body weight, AGD, reproductive and non-reproductive organ weights, age of preputial separation, and serum testosterone levels were unaffected by perinatal DINP treatment. Perinatal DINP treatment significantly induced areolas in male offspring when analyzed on a litter or individual basis (approximately 20% of DINP-exposed males had areolas as compared to 0% in the controls).

Permanent nipples were noted in 2/52 males (2/14 litters). DINP induced a significant level (7.7%) of male reproductive malformations on an individual animal basis (4/52 pups, $p < 0.05$) or litter basis (3/14; $p < 0.06$) when compared to the controls (0/80 individuals and 0/19 litters). The type and severity of the malformations was reported to be highly

variable among individuals. Abnormalities observed in DINP offspring included small and atrophic testes; flaccid, fluid-filled testes; unilateral epididymal agenesis with hypospermatogenesis; and scrotal fluid-filled testis devoid of spermatids.

7. Perinatal Oral Exposure Study of Effects on the Development of Rat Endocrine/Reproductive Systems (Masutomi et al. 2003)

Masutomi et al. (2003) evaluated developmental effects in the offspring of female Sprague-Dawley rats exposed to DINP (CASRN 28553-12-0) in the diet from GD 15 to PND 10 in a non-GLP compliant study (as noted by the study authors). The period of exposure was selected because it is the most sensitive developmental interval for brain sexual differentiation. DINP was tested using a soy-free basal diet to eliminate potential phytoestrogenic effects and was provided to test animals (5/dose) at concentrations of 0, 400, 4,000, or 20,000 ppm. Dosing was terminated on PND 10 and all dams were provided with the soy-free basal diet without added DINP for the remainder of the lactation period. The litters were culled randomly to reserve 5–8 pups. On PND 21, the offspring were weaned, and 5 pups/sex/dose (1 male and 1 female per litter) were reserved for prepubertal necropsy on PND 27, and 8 pups/sex/dose (at least 1 male and 1 female per litter) were reserved for examination of adult-stage animals at postnatal week (PNW) 11. The weanlings were provided with a soy-containing commercial feed without added DINP. Offspring were evaluated for AGD, prepubertal organ weight (brain, adrenals, testes, ovaries, uterus), age and weight at onset of puberty, estrous cyclicity, volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA), and organ weights and histopathology of endocrine organs (brain, pituitary, adrenals, testes, prostate, ovaries, uterus) at PNW 11.

The administered dietary concentrations corresponded to average daily maternal doses during gestation of approximately 0, 30.7, 306.7, or 1,164.5 mg/kg/day for the 0, 400, 4,000, or 20,000 ppm diets, respectively, as estimated by the study authors. The estimated average daily doses during lactation were 66.2, 656.7, and 2,656.7 mg/kg/day, respectively. Maternal toxicity was evident as decreased body weight gain and reduced food consumption at 20,000 ppm. Data for absolute maternal body weights were not reported. Maternal body weight gain was significantly decreased at 20,000 ppm during gestation (GD 15–20; −55%) and lactation (PND 2–10; −85%). Maternal feed consumption was

significantly decreased during gestation (−28%) and lactation (−22%). There were no significant effects on the number of live offspring or on pup body weight or AGD on PND 2 at any DINP concentration when compared to control values. Dose-related decreases were observed in body weight gain of male and female pups on PND 2–10 and were statistically significant at 20,000 ppm (decreases of 55% each for males and females). At the prepubertal necropsy on PND 27, dose-related decreases in body weight were evident in males and were statistically significant at 4,000 (−18%) and 20,000 (−43%) ppm. Body weight in females was significantly decreased (−39%) at 20,000 ppm. Feed consumption data were not reported.

Statistically significant changes were observed for decreased absolute brain weight (males and females), increased relative brain weight (males and females), increased relative adrenals weight (females), decreased absolute and relative testes weight (males), decreased absolute ovaries weight (females), and decreased absolute uterus weight (females). The volume of the SDN-POA in prepubertal animals was unaffected by exposure to DINP. In animals maintained until PNW 11, body weight gain was significantly reduced (−18%) in 20,000 ppm males for PND 21–42, although the response was not dose-dependent. Body weight of exposed females was comparable to the controls. Exposure to DINP had no effect on age at onset of puberty or estrous cyclicity. Body weight at the onset of puberty was significantly reduced in males (−19%) and females (−18%) at 20,000 ppm. Although dose-related decreases (≤9%) in terminal body weight were noted in males and females, the response was not statistically significant for either sex. No significant differences between treated and control animals were observed for absolute or relative endocrine organ weights. Minimal to slight degeneration of stage XIV meiotic spermatocytes and vacuolar degeneration of Sertoli cells were observed in the testes of 4/5 20,000 ppm males compared to an incidence of 0/5 in control males. Scattered cell debris was observed in the epididymal ducts of affected males. Minimal to slight increase of secondary follicles/decrease of corpora lutea was observed in 4/5 20,000 ppm females compared to 1/5 control females. Morphometric analysis detected a slight but statistically significant decrease in the number of corpora lutea in 20,000 ppm females. These data identify NOAEL and LOAEL values of 400 ppm and 4,000 ppm, respectively, for decreased body weight in male rats at prepubertal necropsy at PND 27. These NOAEL and LOAEL concentrations correspond to maternal doses of 66 mg/kg/day and 657 mg/kg/day during

the lactational period, respectively.

Lactational doses were selected for the NOAEL and LOAEL because the decrements in pup body weight occurred after PND 2 and thus appear to result primarily from exposure of pups to DINP via the milk.

8. Evaluation of Steroidogenesis in Fetal Male Rats Exposed to DINP *in utero* (Borch et. al. 2004)

Borch et al. (2004) examined the hormonal response of male Wistar rat fetuses to *in utero* DINP exposure. Pregnant Wistar rats (8/dose) were exposed during gestation (duration of treatment not reported) to 0 or 750 mg/kg/day DINP (CASRN 28553-12-0) by oral gavage in peanut oil. The dose of 750 mg/kg/day was the same as the dose used in the perinatal reproductive study of male reproductive effects conducted by Gray et al. (2000). Male fetuses were sacrificed on GD 21 and blood and testes were collected for measurement of testicular testosterone content (one male/litter), plasma testosterone and luteinizing hormone (LH) concentrations (plasma pooled from all fetuses in each litter), and *ex vivo* testicular production of testosterone (left testes from two fetuses/litter). No information was provided on whether other fetal or maternal endpoints of toxicity were evaluated. Exposure to DINP significantly reduced fetal testicular testosterone content (approximately 3.5-fold) and *ex vivo* production of testosterone from fetal testes (approximately 3-fold). Reduced plasma testosterone content and increased plasma LH content were observed when compared to the controls, but these responses were not statistically significant. These results indicate that *in utero* exposure to DINP disrupts steroidogenesis in male offspring and suggest a possible MOA for the antiandrogenic effects of DINP on male fetal reproductive tract development observed by Gray et al. (2000).

Although there are currently no mechanistic studies for DINP effects on the development of the male reproductive system, relevant data are available from studies of di(n-butyl) phthalate (DBP), a structurally related phthalate. Pre- or perinatal exposure to DBP impairs androgen-dependent development of the male reproductive tract in rats (e.g., Mylchreest et al. 1998; 2000). Some (but not all) malformations reported in male offspring following treatment of pregnant rats with DBP are similar to those observed for DINP (e.g., effects on the epididymis, retained nipples), suggesting that these compounds may share a

common MOA for some developmental effects. Exposure of pregnant rats to DBP (500 mg/kg/day) during gestation leads to rapid and reversible decreases in the expression of several proteins required for cholesterol transport and steroidogenesis in the fetal testis, resulting in decreased testosterone production (Thompson et al. 2004). These observations are consistent with and support the findings of Borch et al. (2004) showing decreased fetal testicular testosterone content and production in male offspring of female Wistar rats treated with 750 mg/kg/day of DINP during gestation.

9. Disposition of DINP and Its Effects on Sexual Development of the Male Fetus Following Repeated Dosing in Pregnant Rats (Lee et al. 2006).

Di-n-butyl phthalate (DBP), diisononyl phthalate (DINP), and di-2-ethylhexyl adipate (DEHA) have been previously shown to effectaffect the endocrine system and most of the endocrine disruptors have estrogenic or antiandrogenic properties, these chemicals may affect sexual differentiation of the brain. To investigate the neuro-developmental effects on rats, , 8 weeks old Wistar-Imamichi rats were allowed to mate, and gestational day (GD) 0 pregnant females were allowed to deliver pups naturally (day of birth=PND 0), and the litter size was adjusted to eight on PND 5. The dams were fed soy-free diet. From GD 15 to the day of weaning (PND 21), the dams were provided soy-free diet that contained 20, 200, 2,000, or 10,000 ppm of DBP, 40, 400, 4,000, or 20,000 ppm of DINP, or 480, 2,400, or 12,000 ppm of DEHA.

On PND 1, body weight and AGD of pups were measured. On PND 7, pups were sacrificed and their brain and blood tissue were immediately removed. The entire hypothalamus was frozen and used for downstream analysis. Serum testosterone and estradiol concentrations were measured from blood. After maturation, estrous cyclicity of females by vaginal smears during postnatal weeks (PNW) 8-9 and PNW 19-20. Preovulatory gonadotropin surge, proestrus in female rats in PNW 20, and serum LH, FSH, and estradiol levels were quantified. Serum LH, FSH, and testosterone levels were quantified in males on PNW 20. Copulatory behavior tests for both males and females were initiated on PNWs 20-21 for male and female rats.

In the DBP, DINP, and DEHA exposed neonatal rats, body weights were significantly decreased compared with those of the control pups of the corresponding sexes, with the exception of the groups given 20 ppm of DBP for both sexes and 200 ppm of DBP for

females. AGD in male neonates was decreased in a dose-dependent manner by maternal exposure to all phthalates tested. In female neonates, an increase in AGD was observed in DBP- and DINP-exposed animals at the highest doses.

On PND 7 Serum testosterone levels were significantly higher in males than females in the control group while there were no differences in serum estradiol levels between the sexes. Only DINP at 40 ppm and DBO at 200 ppm perinatal exposure significantly decreased estradiol levels in female pups while testosterone and estradiol in either sex did not change. On PND 7, hypothalamic expression of grn mRNA was higher in males and p130 mRNA was higher in females. The expression of grn gene in female pups was increased in higher doses of DBP and all the doses, except for 4,000 ppm of DINP prenatal exposure. Hypothalamic expression of p130 mRNA in males was increased by lower doses (20 and 200 ppm) of DBP and all the doses of DINP. Copulatory behaviors in control rats were normal, but in the DINP an DEHP exposed rats the number of mounts and intromissions decreased significantly while DBP exposure did not have any effect. Ejaculations were decreased significantly in DBP, DINP and DEHA exposed rats and only increased in high dose of DBP (10,000 ppm) exposed rats. Serum LH, FSH, and testosterone levels in the male rats were not affected by phthalate exposure, however the Lorde Quotient (LQ) of the females examined on the proestrous day in PNW 20-21 was significantly decreased in all the animals perinatally exposed to DBP, DINP, and DEHA in a dose dependent manner.

These results suggest that inappropriate expression of grn and/or p130 genes in the brains of male and female neonatal rats by perinatal exposure to DINP may exert permanent effects on the hypothalamus, thereby decreasing sexual behavior after maturation.

10. Effects of Maternal Exposure to Di-isononylphthalate (DINP) and 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on Steroidogenesis in the Fetal Rat Testis and Adrenal Gland (Adamsson et al. 2009)

Exposure to DINP shows demasculinization with female-like nipples, small and atrophic testis and epididymal agenesis. In utero exposure to DEHP, DBP and DINP have been shown to disrupt fetal male rat steroidogenesis. 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p*_-DDE), metabolite of DDT (1,1,1-trichloro- 2,2-bis [*p*-chlorophenyl]ethane),

accumulates in body fat. Antagonizing effect of *p,p_*-DDE on AR function is known in both *in vivo* and *in vitro*. *In utero* and lactational exposure to *p,p_*-DDE results in reduced anogenital distance and retained thoracic nipples of male rats; both indicators of prenatal antiandrogen activity. To investigate if exposure to antiandrogens DINP and *p,p_*-DDE during the critical developmental window (i.e. sexual differentiation) can permanently demasculinize the male phenotype by effecting the rat testicular and adrenal steroidogenesis. Pregnant Sprague–Dawley pregnant rats (285–300 g on ED 13.5) were randomly assigned to treatment groups of 7&8 animals for DINP and treated once daily by oral gavage with 250 mg/kg or 750 mg/kg of DINP or with *p,p_*- DDE (5 & 6 animals) at maternal doses of 50 mg/kg) or 100mg/kg from EDs 13.5 to 17.5. Control animals received vehicle (corn oil) only (DINP control: 8 dams; *p,p_*-DDE control: 7 dams). were exposed to DINP (250 and 750 mg/kg) or *p,p_*-DDE (50 and 100mg/kg) respectively from embryonic day (ED) 13.5 to 17.5. The analysis of fetal male rat testicular testosterone (T) content, plasma corticosterone and luteinizing hormone (LH) levels and the expression levels of selected steroidogenic enzymes, regulatory factors and androgen receptors were carried out on ED 19.5.

DINP or *p,p_*-DDE exposure *In utero* did not produce any signs of overt toxicity in the dams. On ED 19.5 the body weights of male fetuses were increased in the group treated with 250 mg/kg DINP (2.65±0.04 g vs. 2.49±0.02 g in control; $p < 0.05$); *p,p_*-DDE had no effect on body weight of male or female fetuses. Corticosterone, increased at 250 mg/kg DINP (313±32 ng/ml vs. 221±33 ng/ml in control), but the increase did not reach statistical significance, p,p-DDE also did not have any significant effect. *In utero* exposure either to DINP or *p,p*-DDE did not alter testicular T levels significantly. RT-PCR performed on testicular and adrenal samples of DINP-treated male fetuses revealed a statistically significant increase in testicular transcript levels of *P*450*scc* at 750 mg/kg and an increase in *StAR*, 3_-*HSD* or *SF*-1 mRNA levels with both doses, a significant increase in testicular *GATA*-4 and *Insl*-3 mRNA levels, at 750 mg/kg. However, DINP exposure had no effect on adrenal *StAR*, *P*450*scc*, 3_-*HSD*, *SF*-1 or *GATA*-4 transcript levels on ED 19.5. *In utero* exposure to DINP did not affect protein levels of testicular StAR, P450scc or 3_-HSD significantly, although 250 mg/kg DINP tended to increase the expression levels of StAR and steroidogenic enzymes Maternal exposure to *p,p_*-DDE had no effect on fetal testicular

or adrenal StAR, p450scc, 3_-HSD or AR protein levels. There were no significant changes in light and electron microscopic observations of maternally DINP-exposed fetal adrenals in contrast to the *p,p_*-DDE exposure.

The study suggests that prenatal treatment of DINP from EDs 13.5 to 17.5 does not down-regulate the activity of steroidogenesis in 19.5-day-old fetal male rat demonstrating that phthalate esters do not act through ARs.

11. Reproductive and Behavioral Effects of Diisononyl Phthalate (DINP) in Perinatally Exposed Rats (Boberg et al. 2011)

In a developmental toxicity study, 80 time-mated 3-day, pregnant Wistar rats (~200 gm body weight), The dams were randomized into five
groups of 16 with similar body weight distributions and housed in pairs until GD 21. The animals were dosed with vehicle (corn oil), 300, 600, 750 or 900 mg/kg/day DINP via oral gavage from GD 7 to PND 17. On GD 21, 4 dams per group were euthanized and fetuses were removed and decapitated. Testes were removed and sampled for histopathology, measurement of testosterone production ex vivo, or measurement
of testosterone content. One testis per litter was subjected histopathology and immunohistochemistry. Blood was collected from all male pups and testosterone levels were quantified. After birth (PND 1), dams and all live pups in the litter were weighed. Pups were sexed and the anogenital distance (AGD) was measured. At PND 13 all pups were weighted and examined for the presence of nipples/areolas, Maternal pup retrieval was measured in all groups. At weaning (PND 21), pups were divided into two subgroups and sexual maturation was determined appropriately. Tail blood was collected on PND 22 for Inhibin B analysis. 1–7 males per litter were euthanized and subjected to autopsy on PND 90. Males were examined for presence of nipples, penile malformations and testicular descent and hormones were analyzed from blood. The female litters were also subjected to autopsy and female parameters were quantified. Motor activity levels and habituation of the rat offspring were recorded at 4 weeks (PND 27/28) of age and in adults (PND 11–12 weeks old). 2–3 months old pups were subjected to Morris maze learning and memory studies,
All dose groups showed effects on testicular histology, particularly all animals were affected in the two highest dose groups (750 and 900 mg/day). Reduced testicular testosterone production *ex vivo* was seen in the DINP exposed groups, but not statistically significant (p

= 0.08, 0.11 and 0.07 in the three highest dose groups, respectively). Testicular testosterone content was significantly reduced in the group exposed to 600 mg/kg bw per day of DINP (p = 0.044) and a slight but not statistically significant reduction was seen in the highest dose group (p = 0.099), However, plasma testosterone or LH levels did not change significantly. Maternal body weight and weight gain during pregnancy was not affected with no change in gestation length, post-implantation loss, litter size, sex ratio or perinatal loss were seen by treatment with DINP. Anogenital distance in male pups on PND 1 was reduced dose dependently by DINP compared to controls and no change in female anogenital distance or birth weight were observed. Statistically significant and dose-dependent increase in nipple retention in male pups was seen at 750 and 900 mg/kg bw. At PND 90, there were no genital malformations and nipple retention between the groups. There were also no significant changes in the weights of livers, thyroids and reproductive organs of males and females rats by DINP treatment. Though, histology of male reproductive organs at PND 90 was not altered by DINP treatment, a dose-dependent reduction in the percentage of motile sperm was seen from 600 mg/kg bw per day. There were no significant changes in the Inhibin levels after DINP treatments, suggesting that the Sertoli cell were functionally normal. The study authors find similar dose-related effects (nipple retention, reduction of anogenital distance, disruption of semen quality) of DINP as previously seen with DEHP and DBP clearly support that DINP is an anti-androgen and a reproductive toxicant but is less potent than DEHP and DBP. Furthermore, the study supports the previously reported findings of persistent malformations in DINP exposed males. The authors identified NOAEL of 300 mg/kg bw and a LOAEL of 600 mg/kg bw per day based on reproductive toxicity and anti-androgenic effects for DINP.

12. Dose-Response Assessment of Fetal Testosterone Production and Gene Expression Levels in Rat Testes Following *in utero* Exposure to Diphthalates (Hannas et al. 2011)

Cumulative toxicity of anti-androgens, including phthalates has been predicted by dose-additive mathematical models. To predict a dose-additive response, the relative potency of each compound in the mixture must be accounted .To identify the potency factor for disrupting fetal testis function as needed for cumulative risk assessment, a dose-response

data was required to build the relationship between several individual phthalates and fetal testicular T production and/or gene expression to characterize their relative potency, To identify the dose response and potency information for fetal testicular T production and gene expression pregnant rats SD and W pregnant rats were dosed with increasing concentrations of individual phthalates.

Charles River SD and W timed pregnant rats (3–6 dams per dose group) were dosed daily on GDs 14–18 by oral gavage with vehicle (corn oil) or 100, 300, 500, 625, 750, or 875 mg DEHP/kg/; 4 dams per dose group with vehicle (corn oil) or 100, 300, 600, or 900 mg DIHP/kg/day; Harlan SD dams (3 dams per dose group) corn oil, 100, 300, 600, or 900 mg DIBP/kg/day; 3–6 dams per dose group with corn oil (control), 500, 750, 1000, or 1500 mg DINP/kg/day (CAS 28553-12-0) and 3 dams per dose with DINP from Sigma (CAS 68515-48-0, lot 03005TR. Harlan SD dams were dosed orally on each of GDs 14–18 with the vehicle (0%) or one of five dilutions of a mixture of (1) DEHP, (2) DIHP, (3) DIBP, (4) DBP, (5) BBP (CAS 85-68-7, lot 03405JH),( 6) dicyclohexyl phthalate (DCHP; CAS 84-61-7, lot 17518JB), (7) di(n)heptyl phthalate (D(heptyl)P; CAS 3648-21-3, lot 125AG), (8) di-n-hexyl phthalate (D(hexyl)P; CAS 84-75-3, lot 139AG), and (9) DPeP. The dosage levels were 100, 66.67, 33.33, 16.66, and 8.325%. The mixture ratio of the phthalates was designed such that each phthalate would contribute equally to the effects of the mixture if the phthalates behaved in a dose-additive manner.

Maternal body weight gain was significantly decreased at doses of 625 mg DEHP/kg/day and above for both strains ($p < 0.02$) suggesting that body weight decline was similar for the two strains over the 5-day dosing regimen. DEHP reduced ex vivo T production from fetal testes in a dose-responsive manner, with significant reductions observed at 300 mg DEHP/kg/day and higher in both the SD and W strains ($p<0.0001$). T production levels were lower in SD strain compared to W strain in both control and 100 mg/kg DEHP treated group ($p < 0.01$ and $p < 0.03$, respectively). DEHP treatment reduced fetal testis insl3 mRNA expression in both strains ($p < 0.0001$ for dose effect) in a dose-dependent manner with significant reduction in insl3 mRNA expression at 625 mg DEHP/kg/day and greater in the SD rat and at 500 mg DEHP/kg/day and greater in the W rat ($p < 0.05$), though overall reduction in insl3 mRNA levels did not differ between strains. The mRNA expression of the androgen synthesis genes StAR and Cyp11a also were significantly reduced at doses of 500 mg DEHP/kg/day and greater in both strains ($p < 0.0005$ and $p < 0.0001$, respectively.

Treatment with DIHP, DIBP, or DINP did not induce maternal mortality, overt toxicity, or reduce maternal body weight or reduce litter size at any dosage level tested. Addition of hCG significantly increased fetal testicular T production in each dosage group by about 2.3-fold for DIHP. DIBP significantly reduced fetal testicular T production at doses of 300 mg/kg/day or greater ($p < 0.005$) and was equipotent to DIHP and DEHP. Both DINP formulations reduced fetal testicular T production similarly in a dose-responsive manner at doses of 500 mg DINP/kg ($p < 0.05$) or greater ($p < 0.01$). DIBP reduced fetal testis RNA expression levels for StAR at dosage levels of 300 mg/kg/day and greater ($p < 0.05$) and Cyp11a levels at 100 mg/kg/day and greater ($p < 0.05$). DINP in both formulations, reduced StAR and Cyp11a levels, but at higher dosage levels than DIBP. The nine phthalate mixtures did not significantly reduce maternal weight gain or fetal viability at any dilution of the top dose of 650 mg/kg/day phthalates. However, the mixture induced significant reductions in fetal testicular T production at doses of 17% and greater ($p < 0.01$). T production was also reduced at 8% of the top dose, the lowest dose tested by about 15%; however, this effect did not attain statistical significance ($p ¼ 0.15$ using log10 transformed T production and $p < 0.03$). In the phthalate mixtures tested, there was an additive effect on reduced fetal T production in a dose-dependent manner as predicted by dose addition.

13. A Short-Term *in vivo* Screen Using Fetal Testosterone Production, a Key Event in the Phthalate Adverse Outcome Pathway, to Predict Disruption of Sexual Differentiation (Furr et al. 2014)

Furr et al. evaluated 27 chemicals, including DINP, for disruption of fetal testosterone synthesis in a short-term *in vivo* screening study. Overall, the 27 chemicals were tested over a 2-3 year period in 66 blocks, with approximately each block consisting of 15 pregnant Charles River SD rats. Herein, only results for DINP are summarized. Pregnant SD rats were administered 0 (corn oil vehicle control) or 750 mg/kg/day DINP via gavage on GDs 14-18 (N = 5 control dams and 3 DINP treated dams). Dams were weighed daily throughout the exposure period. Dams were sacrificed on GD 18 approximately two hours after the final treatment. Fetal viability was then determined and fetal testes were collected for determination of *ex vivo* fetal testis testosterone production. In the first experiment (block 1), treatment with DINP (CASRN 68515-48-0) did not affect fetal viability or dam weight gain but resulted in a 24% reduction in *ex vivo* fetal testis testosterone production. However,

this result was considered equivocal because when the data was $\log_{10}$ transformed, the resulting change in testosterone production was not statistically significant. Because results for DINP were equivocal, the experiment was repeated in block 5. In the second experiment, DINP did not affect fetal viability or dam weight gain, however, in fetal testis testosterone production was significantly reduced 38%. Furthermore, when results were pooled from animals treated with DINP in blocks 1 and 5, fetal testis testosterone production was found to be significantly reduced by 750 mg/kg/day DINP.

An additional experiment using the same protocol described above was conducted with 750 mg/kg/day DINP (CASRN 28553-12-0) in block 7. Similar to previous experiments with DINP (68515-48-0), no effect on fetal viability or dam weight gain were reported. In this experiment, a statistically significant 50% reduction in *ex vivo* fetal testis testosterone production was observed.

c) Conclusions for Reproductive and Developmental Toxicity

The available data for developmental toxicity ([ REF _Ref99637756 \h * MERGEFORMAT ]) generally shows a consistent pattern of effects within study type. The results of the one- and two-generation reproductive studies indicate that DINP affects postnatal growth, as evident from significantly reduced pup growth at doses of 143–285 mg/kg/day (during gestation and lactation). The results of two developmental toxicity studies on DINP (Hellwig et al. 1997; Waterman et al. 2000) are also consistent. In both studies, DINP exposure *in utero* resulted in increased incidences of rudimentary lumbar and/or supernumerary cervical ribs and adverse renal effects in fetuses.

Hellwig et al. (1997) identified NOAEL and LOAELs of 200 and 1,000 mg/kg/day, respectively, for these developmental effects. EPA has identified lower NOAEL and LOAEL values of 100 and 500 mg/kg/day, respectively, based on effects observed in the developmental study conducted by Waterman et al. (1999). DINP causes malformations of the reproductive tract and alterations in fetal testicular testosterone production and content in male offspring of rats exposed to 750 mg/kg/day during gestation (Gray et al. 2000; Borch et al. 2004).

EPA believes that the weight of evidence from the available reproductive and developmental toxicity studies that were considered and presented in [ REF _Ref99637756 \h

* MERGEFORMAT ] suggests that DINP causes adverse developmental effects in animals. The adverse effects include decreased body weight of pups during lactation in a rat two-generation reproductive toxicity study and in a multi-dose perinatal exposure study; adverse renal and skeletal effects observed in two rat developmental toxicity studies; altered sexual differentiation observed in a single dose gavage study (750 mg/kg/day) of perinatally-exposed male rats; and occurrence of histological lesions in the ovaries and testes of male and female rats exposed perinatally via the diet (1,164–2,656 mg/kg/day).

Reduction in the mean body weight of pups exposed to DINP either for one generation, two generations, or perinatally is a sensitive indicator of developmental toxicity, in part because it is a continuous variable. The Agency believes that the weight of evidence indicates reduced pup body weight is a serious effect because (1) the observed responses were statistically significant; (2) the responses were dose-related, (3) the reductions ranged from 9–43% below control values (a range that is consistent with biological significance); (4) the magnitude of the response tended to increase with DINP exposure over time via lactation exposure during the post-natal period; (5) the reductions were observed in both sexes and in both F1 and F2 generations of the two-generation study; (6) the weight reductions were noted in both one- and two-generation and perinatal exposure studies; and (7) the response may have long-term consequences. Although there is always a question as to whether weight reduction is a permanent or transitory effect, little is known about the long-term consequences of short-term fetal or neonatal weight changes; however, previous study has shown that exposure to chemicals during organogenesis that reduced pup birth weight also permanently reduced adult mouse weight with about 50% of the chemicals (about 40 tested) (Gray et al.,1984). Therefore, the Agency has concerns for potentially serious developmental effects of DINP in humans.

EPA believes that the kidney and skeletal variations observed in rats treated with DINP are serious because they are structural effects that indicate that development has been disrupted. The observed renal effects and skeletal variations occurred in the absence of or at minimal maternal toxicity. In particular, the occurrence of extra cervical ribs may be of serious health consequence. As noted by NTP-CERHR (2000), supernumerary cervical ribs are an uncommon finding, and their presence may indicate a disruption of gene expression leading to this structural anomaly. In addition, there is concern that cervical ribs may interfere with normal nerve function and blood flow.

EPA believes that the effects on sexual differentiation observed in male rats by Gray et al. (2000) are serious because they represent gross morphological malformations not normally seen in development of this species. The discrepancy between the antiandrogenic effects observed in the perinatal exposure study (Gray et al. 2000) and the absence of similar effects in the two-generation reproductive study conducted by Waterman et al. (2000) may be explained, in part, by the dose (750 mg/kg) used by Gray et al. (2000) and by differences in the protocol used for each study. Exposures during gestation in the two-generation study did not reach the dose that was used in the Gray et al. (2000) perinatal exposure study during gestation (approximately 560 mg/kg/day vs. 750 mg/kg/day, respectively) and the reproductive parameters affected in the study by Gray et al. (2000), including nipple retention, anogenital distance, age at testes descent, and age at preputial separation, were not measured in the two-generation reproductive study.

Furthermore, the number of F1 animals examined by Waterman et al. (2000) was not sufficient to detect the low (7.7%) but statistically significant incidence of malformations observed by Gray et al. (2000). The perinatal exposure study reported by Masutomi et al. (2003) did not detect the same type of alterations reported by Gray et al. (2000), although the administered dietary concentrations resulted in doses (306.7–656.7 mg/kg/day and 1,164–2,657 mg/kg/day) that bracketed the single gavage dose of 750 mg/kg/day administered by Gray et al. (2000). However, Masutomi et al. (2003) examined fewer litters (5 vs. 14), likely examined fewer pups (number of pups and developmental endpoints examined prior to culling were not reported) and did not report use of the same type of detailed internal and external examinations used by Gray et al. to detect areolas, retained nipples, and other developmental effects. In addition, it is possible that the differing routes of administration (gavage vs. diet) used in these studies may have resulted in different peak blood concentrations of DINP.

Although the study by Gray et al. (2000) used a single dose and a NOAEL/LOAEL could not be established, the observed effects indicate that DINP has the potential for antiandrogenic effects in neonatal male rats when tested at 750 mg/kg/day. The effects of DINP on sexual differentiation were characterized by the study authors as malformations for the tested species and are therefore believed to be permanent (i.e., not transient or reversible) and adverse. The observed effects may have resulted from inhibition of fetal testis hormone production during sexual differentiation, a process that is critical in all mammals including

humans. It has been demonstrated that several other structurally related phthalate esters (DBP, DEHP, and BBP) also alter sexual differentiation and do so by altering fetal testis testosterone production and/or content (Parks et al. 2000; Thompson et al. 2004) and insulin-like hormone 3 (Insl3) production (Wilson et al. 2004), resulting in malformations of male reproductive tissues that require these hormones for development. The results of a recent study by Borch et al. (2004), which showed decreased fetal testis production and content of testosterone in offspring of female rats treated with DINP during gestation, are consistent with this pattern and increase the weight of evidence for disruption of testosterone synthesis as a potential MOA for the observed effects on the male reproductive system. Although information is currently lacking on (1) the precise mechanism(s) responsible for DINP-induced malformations and its relevance to humans, and (2) the critical window of susceptibility for these effects during reproductive development, the Agency believes that it is premature to conclude that humans would not be affected if exposed to sufficient concentrations of DINP or its metabolites at critical stages of reproductive development.

**Table [ SEQ Table * ARABIC ]. Developmental Effects Observed in Reproductive and Developmental Toxicity Studies of DINP**

| Reference | Description | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Developmental Effect |
|---|---|---|---|---|
| Waterman et al. (2000) | One generation pilot dietary reproductive study in Sprague Dawley (SD) Rats Estimated doses: 0, 377–404, 741–796, 1,087–1,186 mg/kg/day during gestation and 0, 490–932, 1,034–1,731, 1,274–2,246 during lactation | – | 377–932 during gestation and lactation | Decreased offspring body weight at PND 21. |
| Waterman et al. (2000) | Two generation dietary reproductive study in SD rats. Estimated doses: 0, 143–146, 287–288, and 555–560 mg/kg/day during gestation and 0, 254–285, 539–553, and 1,026–1,129 mg/kg/day during lactation | – | 143–285 during gestation and lactation | Decreased pup body weights gain in F1 males and females on PND 21. |
| Waterman et al. (1999) | Developmental gavage study in SD rats. Doses: 0, 100, 500, 1,000 mg/kg/day | 100(a) | 500(a) | Increased incidences of fetuses and litters with dilated renal pelves; accessory lumbar ribs and rudimentary cervical ribs. |

**Table [ SEQ Table * ARABIC ]. Developmental Effects Observed in Reproductive and Developmental Toxicity Studies of DINP**

| Reference | Description | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Developmental Effect |
|---|---|---|---|---|
| Hellwig etal. (1997) | Developmental gavage study of DINP-1,DINP-2, DINP-3 in Wistar rats<br>Doses: 0, 40, 200, 1,000 mg/kg/day | 200 | 1000 | Increased incidences of rudimentary cervical and accessory lumbar ribs; urogenital and skeletal variations with DINP-3. |
| Gray et al. (2000) | Perinatal gavage study in SD rats; evaluateddevelopmental alterations in neonatal males<br>Single dose: 750 mg/kg/day | – | No LOAEL can be established (single dose study) | As neonates, exposed males displayed female-like nipples/areolas, reproductive malformations, and altered sexual differentiation. |
| Masutomi etal. (2003) | Perinatal dietary exposure study in SD rats;utilized soy-free diet to eliminate potential estrogenic effects<br>Estimated doses:0, 30.7, 306.7, or 1,164 mg/kg/day during gestation; 0, 66.2, 656.7,or 2,656 mg/kg/day during lactation | 66 | 657 during lactation | Decreased postnatal body weight in male rats on postnatal day 27<br>Also histopathological changes in offspring testes and ovaries at high dose. |
| Borch et al. (2004) | Gestational exposure study in Wistar rats. Testosterone production of fetal testis measured *ex vivo*; testosterone content measured in testes and plasma; luteinizing hormone concentration measured in plasma. | – | 750 | Decreased testosterone production by fetal testes *ex vivo*. Decreased testosterone content of fetal testes. |
| Lee et al. 2006 | Neuro-developmental study, maternal rats were exposed to (0, 2, 20, 200, 1,000 mg/kg/day) from GD 15 to PND 21.GD exposed rats were re-exposed to DINP after maturation | – | 2 ( LOEL)(a) | In the 2 mg/kg dose group, increase in hypothalamic granulin (grn) and p130 mRNA levels. Males showed less copulatory behavior, lordosis quotient decreased in females. |
| Adamsson et al. 2009 | Short-term developmental exposure study, SD rats were exposed to two doses of DINP (250 and 750 mg/kg) from GD 13.5 to 17.5 days to determine anti androgenicity during the critical developmental stage | | 750(a) | No down-regulation of steroidogenesis ED 19.5 male rat fetus. No changes in testicular and adrenal StAR, P450scc, 3beta-HSD and AR. |
| Clewell et al. (2011a) | Disposition of Diisononyl Phthalate and its Effects on Sexual Development of the Male Fetus Following Repeated Dosing in Pregnant Rats. | 47(a) | 242(a) | The developmental NOAEL and LOAEL were determined based on induction of MNGs and reduced testosterone in fetal testes. |

**Table [ SEQ Table * ARABIC ]. Developmental Effects Observed in Reproductive and Developmental Toxicity Studies of DINP**

| Reference | Description | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Developmental Effect |
|---|---|---|---|---|
| Clewell et al. (2011b) | A Dose Response Study to Assess Effects After Dietary Administration of Diisononyl Phthalate (DINP) in Gestation and Lactation on Male Rat Sexual Development | 50(a) | 250(a) | The developmental LOAEL is 3,800 ppm (~250 mg/kg-bw/day) for effects seen in male pups, including reduced pup weight and increased MNGs in the testes at ≥3,800 ppm and decreased AGD and increased incidence and severity of LC aggregation in the testes at 11,400 ppm. The developmental NOAEL is 760 ppm (~50 mg/kg-bw/day). |
| Hannas et al. 2011 | Reproductive developmental study, Harlan SD rats timed pregnant rats exposed from GD 14–18. Fetal testes endpoints are measured fetal T production and testicular gene expression | | 500 | Reduced fetal testicular T production, StAR and Cyp11a gene expression levels. from 500 mg/kg |
| Boberg et al. 2011 | Short-term developmental toxicity study rats were exposed to 0, 300, 600, 750, and 900 mg/kg of DINP from GD 7–17 to estimate fetal testosterone production, testicular histopathology, postnatal development, reproductive organs, semen quality, and behavior. | 300 (a) | 600 | Nipple-retention, reduced anogenital distance, and disruption of semen quality at 600 mg/kg |
| Furr et al. 2014 | Short-term study, CD-1dams were exposed to 750 mg/kg from GD 14–18 and *ex vivo* testis testosterone production (T Prod) was measured on GD 18 | | Single dose study | Testis endocrine function *in utero*, development of male rat reproductive tract lesions and reproductive problems. |
| (a) As determined by EPA after review of the study data. | | | | |

## IX. Other Relevant Data

1. Responses to DINP Exposure in PPAR Alpha-Deficient Mice (Valles et al. 2003)

Valles et al. (2003) conducted four studies in wild-type and PPAR alpha-deficient mice to investigate the role of PPAR alpha in responses to DINP ([ REF _Ref99637840 \h * MERGEFORMAT ]). In Study 1, male and female B6C3F1 mice (9-10 weeks of age) were fed diets containing 0, 150, 1,500, 4,000, or 8,000 ppm of DINP (CASRN 68515-48-0) for 2 weeks. In Study 2, male and female wild type and PPAR alpha-null mice (approximately 30

weeks of age) and male and female B6C3F1 mice (approximately 26 weeks of age) were fed 0 or 8,000 ppm of DINP (CASRN 28553-12-0) for 1 or 3 weeks. Older mice were used in Study 2 because younger mice were not available. In Study 3, male and female wild type, PPAR alpha-null, and B6C3F1 mice (9 weeks of age) were fed 0 or 8,000 ppm of DINP (CASRN 68515-48-0) for 1 week. In Study 4, male wild-type and PPAR alpha-null mice were given 0 or 1,000 mg/kg gavage doses of DINP (CASRN 68515-48-0) in 0.1% methylcellulose daily for 7 days.

Exposure to DINP caused statistically significant, dose-related increases in liver-to-body weight ratios in male B6C3F1 mice exposed to 4,000 or 8,000 ppm and females exposed to 1,500 ppm and higher in the diet for 2 weeks (Study 1). Exposure to DINP induced significant increases in liver-to-body weight ratio in male SV129 wild type mice exposed to 8,000 ppm for 1 or 3 weeks (Studies 2 and 3), but not in male PPAR alpha-null mice. Younger (10–12 weeks old) female B3C6F1 (Study 1) and SV129 mice (Study 3), but not younger female PPAR alpha- null mice (Study 3) had increased liver-to-body weight ratios following dietary exposure to DINP. In contrast, increased liver-to-body weight ratios were evident both in 30-week-old female wild type (B6C3F1 and SV129) and PPAR alpha-null mice exposed to 8,000 ppm of DINP for 3 weeks (Study 2), indicating that the increase in relative liver weight was independent of PPAR alpha in older female mice. Liver weight was unaffected in wild type and PPAR alpha-null mice administered DINP for 7 days by gavage (Study 4). Known peroxisome inducers (diethylhexyl phthalate, WY-14,643) gave positive results in parallel experiments with wild type mice, suggesting that the tested dose of DINP may have been too low. Hepatocyte proliferation was measured in female mice from Study 3. Female B6C3F1 mice exposed to DINP showed significantly increased (approximately 2- to 3-fold) bromodeoxyuridine (BrdU) labeling index (LI) in periportal and centrilobular zones of the liver, but not the mid-zone, when compared to the controls. The LI was significantly increased (approximately 2-fold) in the centrilobular zone in female SV129 wild-type mice after DINP exposure. No changes in hepatocyte labeling index were evident in PPAR alpha-null mice following DINP exposure. DINP increased palmitoyl-CoA oxidase activity in the livers of male and female B6C3F1 mice exposed to 4,000 or 8,000 ppm (Studies 1 and 3) and in SV129 wild-type mice exposed to 8,000 ppm for 1 or 3 weeks (Study 3). Palmitoyl-CoA oxidase activity was not increased in PPAR alpha-null mice treated with 8,000 ppm DINP for up to 3 weeks. In Study 3, the protein levels of four enzymes involved in

β- and ω- oxidation of fatty acids (acyl CoA oxidase, multifunctional protein II, thiolase, Cyp4a) were increased in male and female wild type mice when exposed to DINP. Induction of Cyp4a was completely abolished in livers isolated from male and female PPAR alpha-null mice exposed to DINP. Reduced but detectable induction of the remaining proteins was observed in male and female PPAR alpha-null mice; a possible explanation for this response is weak induction through other PPAR subtypes. Overall, these data support the hypothesis that PPAR alpha mediates multiple physiological and biochemical responses following DINP exposure.

Five genes involved in DNA repair and metabolism (ATP-dependent DNA helicase II), drug metabolism (Cyp2a4), and protein trafficking (FKBP-1, FKBP-13) were up-regulated, while one gene involved in DNA repair and metabolism (Endonuclease III Homolog 1) was down-regulated, in wild type mice, but not PPAR alpha-null mice, exposed to 8,000 ppm DINP for 3 weeks. The biological and toxicological significance of these changes in gene expression is unknown.

**Table [ SEQ Table * ARABIC ]. Overview of Studies Conducted by Valles et al. (2003)**

| Study | CASRN | DINP Dose | Duration (weeks) | Age of Mice (weeks) | Tested Strains | | |
|---|---|---|---|---|---|---|---|
| | | | | | $B6C3F_1$ | SV129 Wild Type | PPAR alpha-null |
| 1 | 68515-48-0 | 0 ppm | 2 | 9–10 | X | | |
| | | 150 ppm | 2 | 9–10 | X | | |
| | | 1,500 ppm | 2 | 9–10 | X | | |
| | | 4,000 ppm | 2 | 9–10 | X | | |
| | | 8,000 ppm | 2 | 9–10 | X | | |
| 2 | 28553-12-0 | 0 ppm | 1 | 26–30 | X | X | X |
| | | 8000 ppm | 1 | 26–30 | X | X | X |
| | | 0 ppm | 3 | 26–30 | X | X | X |
| | | 8000 ppm | 3 | 26–30 | X | X | X |
| 3 | 68515-48-0 | 0 ppm | 1 | 9 | X | X | X |
| | | 8000 ppm | 1 | 9 | X | X | X |
| 4 | 68515-48-0 | 0 mg/kg/day | 1 | 9 | | X (males only) | X (males only) |
| | | 1000 mg/kg/day | 1 | 9 | | X (males only) | X (males only) |

2. Assessment of Peroxisome Proliferation in Mice at DINP Doses Observed to Induce Hepatic Tumors (Kaufmann et al. 2002)

Kaufmann et al. (2002) examined the effect of DINP (CASRN 68515-48-0) on indicators of peroxisome proliferation in male and female B6C3F1 mice (5–8/sex/dose) exposed to DINP in the diet for up to 4 weeks. The specific objective of the study was to obtain dose-response data for peroxisome proliferation for comparison with dose-response data for hepatic tumorigenesis in the two-year cancer bioassay in mice reported by Moore (1998b). The dietary concentrations tested (0, 500, 1,500, 4,000, and 8,000 ppm) were those used in the cancer bioassay. The endpoints evaluated included liver weight (1 and 4 weeks); peroxisomal volume density (4 weeks); induction of peroxisomal enzyme activity (cyanide-insensitive palmitoyl-CoA oxidase; 4 weeks); DNA replicative synthesis (1 and 4 weeks); and rates of apoptosis (1 and 4 weeks).

The dietary concentrations of 0, 500, 1,500, 4,000, and 8,000 ppm used in this study corresponded to average daily doses of approximately 0, 100, 300, 800, or 1,600 mg/kg/day as calculated by the study authors. Statistically significant increases were observed in relative and absolute liver weights in males and females at 4,000 ppm and above at both the 1- and 4-week sacrifices, while at 1,500 ppm, absolute (but not relative) liver weight was increased in males and relative (but not absolute) liver weight was increased in females at 4-week sacrifices. Statistically significant increases were also observed in numbers of peroxisomes (all treated males and females at 1,500 ppm and above); peroxisomal volume (males and females at 1,500 ppm and above); peroxisomal enzyme activity (all treated males; females at 1,500 ppm and above); hepatic cell labeling index (all treated males and 4,000 and 8,000 ppm females at the 1-week time point); and apoptosis (8,000 ppm males and 15,00 ppm females at week 1). These data can be compared to the effective concentrations in the Moore (1998b) study, where DINP resulted in significantly increased incidences of hepatic tumors in male mice at concentrations of 4,000 ppm and above and in female mice at concentrations of 1,500 ppm and above.

Kaufmann et al. (2002) concluded that their dose-response data conclusively demonstrate that peroxisome proliferation is the MOA for DINP-induced liver cancer in B6C3F1 mice. However, it should be noted that, although these data clearly demonstrate induction of peroxisome proliferation in male and female mice by DINP, the pattern of dose response does not always correlate closely with the tumor response data. For example, the

incidence of hepatic tumors was significantly increased at a lower concentration of DINP in females than in males, while indicators of peroxisome proliferation were in most cases significantly increased in males at dietary concentrations equal to or lower than observed to elicit the same type of response in females. In addition, significantly increased levels of hepatic cell proliferation were observed at much lower concentrations in males (500 ppm) than in females (8,000 ppm). Overall, the results of this study do not appear to fully support the study authors' conclusions regarding dose- response and MOA for DINP-induced hepatic tumors.

3. Consideration of Human Relevance of Liver Effects

Induction of peroxisome proliferation by phthalates in the liver as well as increased liver weight in rats and mice has been proved to be unquestionable well documented. In some cases, liver cancer was also observed following longer-term oral administration of high doses of phthalates. It is well established that the peroxisome proliferator-activated receptor (PPAR) alpha plays a role in peroxisome proliferation-induced liver effects (Corton and Lapinskas 2005) by sustained activation of beta-oxidation system resulting in uncontrolled reactive oxygen species (ROS) and alteration of the biological oxidation-reduction system. However, the relevance of the hepatotoxic effects of phthalates observed in rodents is difficult to establish due to the species-specific differences in the peroxisomal proliferation response (rodents being significantly more sensitive than humans to PPAR alpha-mediated induction of peroxisome proliferation) (ECB 2008; NICNAS 2010; US CPSC 2010a), though human and rodent PPAR alpha were shown to be equally potent in inducing pleotropic responses of peroxisomal proliferators (Yu et al. 2001).

Recently *in vitro* genomic experiments have clearly shown that mouse and human hepatocytes upregulate the same oxidative networks of fatty acid transport and metabolism (Mcmullen et al. 2014). Interestingly, in the *de novo* transcription factor binding motif analysis the representative DNA sequence associated with rat PPARα binding (the peroxisome proliferator response element [PPRE]) was similar to the sequence associated with human PPARα binding, implying that rat or human PPARα should bind equally well to either to a rat or a human PPRE and was very similar in all the genes that were upregulated by the activation of PPAR irrespective of the agonist and only differed in the sequence identity in the down regulated genes. Activation upregulating signaling network, may not be

sufficient to cause proliferative response. Rodents have a secondary pathway controlled by PPARα-response elements that lack conservation of nucleotides in PPRE flanking regions (McMullen et al. 2020 and Chandra et al. 2008).

Several recent studies have suggested that the mechanisms of liver toxicity of peroxisome proliferators have not been entirely elucidated and that multiple pathways may exist, some that are likely PPAR alpha-independent such as constitutively activated receptor (CAR) (Ito et al. 2007; Yang et al. 2008; Eveillard et al. 2009; Ren et al. 2010), hCAR2 was shown to be specifically upregulated by metabolites of DEHP and DINP (Laurenzana et al. 2016). Based on this, liver effects cannot be precluded as an effect potentially relevant to humans and should be included in the characterization of health effects of DINP. Cumulative evidence implicates that hyperactivation of PPAR induced by peroxisome proliferators plays a central role in hepatocarcinogenesis by disproportionate increases in $H_2O_2$-generating enzymes and reductions in $H_2O_2$-degrading enzymes and thus, generating excess ROS resulting in sustained oxidative stress and progressive estrogen receptor (ER) stress with activation of unfolded protein response signaling. The latter coupled with hepatocellular proliferation are the fundamental causes of peroxisome proliferators-induced hepatocarcinogenesis. Basic PPAR signaling mechanisms are intact in all mammals and it is the relative levels of activation of this mechanism account in part to species sensitivity and relative resistance. The idea that PPAR signaling is not relevant and the risk to humans is minimal is erroneous, simplistic, and misleading (Reddy 2004; Misra et al. 2013).

4. Endocrine Studies

The effects of DINP on the aryl hydrocarbon receptor (AhR), androgen receptor (AR) and estrogen receptor (ER) activity using the luciferase reporter gene expression bioassay in mouse Hepa1.12cR cells (AhR-CALUX) and in transient transfected Chinese Hamster Ovary (CHO-K1) cells (AR-CALUX). DINP did not activate or inhibit AhR activity or AR activity up to $1\times10^{-10}$ to $1\times10^{-4}$ M tested (Krüger et al. 2008). The result for AR is consistent with the results of studies by others (Roy et al. 2004; Takeuchi et al. 2005). Takeuchi and colleagues (2005) assessed human ERα, human ERß, and human AR activity using a reporter gene assay in CHO cells and found that DINP did not show any estrogenic/anti-estrogenic or androgenic/anti-androgenic activity up to concentrations of $10^{-5}$ M.

## X. Systemic Effects from Non-apical End Points

A summary of the studies that were available post 2005, were summarized in [ REF _Ref99637906 \h * MERGEFORMAT ]. However, these studies were not used for generation of point of departure (POD) and were only considered as non-apical end points and for hazard screening as there was not enough information available currently for quantifiable hazard assessment.

a) Exposure to Diisononyl Phthalate Induced an Increase in Blood Pressure through Activation of the ACE/AT1R Axis and Inhibition of NO Production (Deng et al. 2019)

Epidemiological studies have found that diisononyl phthalate (DINP) is associated with an increase in blood pressure. In a study to elucidate the underlying mechanism of the observed increases in blood pressure, groups of C57/BL6 mice (8/group) were administered DINP via oral gavage at doses of 0 (saline vehicle control), 0.15, 1.5 or 15 mg/kg/day DINP for 6 weeks, with or without induction of hypertension via dexamethasone (subcutaneous injection of 1 mg/kg/day). In addition to these 8 treatment groups, the study included a high dose DINP group with dexamethasone-induced hypertension concurrently exposed to an angiotensin converting enzyme (ACE) inhibitor, Enalapril maleate, at 5 mg/kg/day via oral gavage for 6 weeks, and a paired control without DINP. At study termination: measurements of systolic blood pressure (SBR), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate were taken; blood samples were collected from the aorta for measurements of serum NO levels; histopathology was conducted on the aorta, heart, and kidneys; and immunohistochemistry analysis of the aorta included ACE, AT1R, and eNOS.

DINP treatment alone resulted in dose-dependent increases in SBP, DBP, and MBP, attaining statistical significance ($p<0.01$) at 1.5 and 15 mg/kg/day DINP; while HR was increased ($p<0.01$) only at 15 mg/kg/day DINP. Specifically, SBP was increased approximately 18% at 15 mg/kg/day (133 ± 2 mm Hg) compared to saline controls (113 ± 2 mm Hg). DEXA treatment alone increased SBP, DBP, MBP, and HR, and when co-treated with DINP, showed more pronounced dose-dependent increases ($p<0.01$) in blood pressure and heart rate. Along with the blood pressure increase, DINP exposure induced pathological changes in the heart, aorta, and kidney. The inter-ventricular septum and the ventricular wall were significantly ($p<0.01$) thickened in the DINP-treated mice, with the ventricular lumen

noticeable smaller, compared to controls. Blood vessels of the DINP-treated mice showed thickening of the blood vessel wall, hyperplasia and hypertrophy of smooth muscle cells, increased extracellular matrix, and accumulation of inflammatory cells compared to controls. In the kidneys, vacuolization, hyaline degeneration in the glomerulus, smaller glomeruli, and thickened glomerular basement membrane were observed with DINP treatment, but co-treatment with ACEI effectively inhibited these lesions. Additionally, DINP exposure alone or with DEXA treatment enhanced the expression of ACE and AT1R, and inhibited eNOS expression and NO production.

b) Perinatal Exposures to Phthalates and Phthalate Mixtures Result in Sex-Specific Effects on Body Weight, Organ Weights, and Intracisternal A-Particle (IAP) DNA Methylation in Weanling Mice (Neier et al. 2019)

The viable yellow agouti ($A^{vy}$) mouse model was used to investigate the effects of perinatal phthalate exposure. Dams (*a/a* genotype) were fed control (7% corn oil, phytoestrogen-free diet) diets and diets containing 25 mg DEHP/kg chow, 25 mg DBP/kg chow, 75 mg DINP/kg chow, 25 mg DEHP/kg chow + 75 mg DINP/kg chow or 25 mg DEHP/kg chow + 75 mg DINP/kg chow + 25 mg DBP/kg chow starting two weeks prior to mating. Study authors reported that these doses corresponded to approximately 5 mg/kg/day for DEHP and DBP, and 15 mg/kg/day for DINP. After two weeks on the experimental diet, dams (*a/a*) were mated with $A^{vy}$/a males. Dams remained on the experimental diet throughout the mating period, gestation, and lactation until pups were weaned on PND 21. 15 to 19 viable litters were produced from the various treatment groups, however, the exact number of mating pairs included per treatment group was not reported. Results from the DINP, DINP + DEHP, and DINP + DEHP + DBP treatment groups are reported in the following paragraph, while results from the DEHP and DBP alone treatment groups are not reported.

Compared to the control, treatment with DINP, DINP + DEHP, or DINP + DEHP + DBP did not significantly alter pup genotype ratio, sex ratio, number of litters, pups per litter, or pup mortality rate through weaning. Treatment with DINP significantly increased the body weight of *a/a* (10.3% increase) and $A^{vy}$/a (19.6%) female pups and $A^{vy}$/a, but not *a/a* (trending increase, but did not reach statistical significance), male pups on PND 21. Treatment with DEHP + DINP resulted in a 12.7% increase in body weight of $A^{vy}$/a male pups on PND 21, however, body weights of female pups and *a/a* male pups were unaffected.

A trend ($p < 0.1$) in increase pup body weight was observed in $A^{vy}$/a and *a/a* females and $A^{vy}$/a males treated with DINP + DEHP + DBP, but these changes did not reach statistical significance. Absolute and relative liver, pancreas, gonadal fat, spleen, kidney and brain weight was determined on PND 21. Organ weight changes were reported by sex, but not by genotype. Absolute (27.7% inc.) and relative (15.5%) liver weight was increased in female pups treated with DINP, DINP + DEHP (24.8% (abs), 17.7% (rel.)), and DINP + DEHP + DBP (21.0% (abs.), 10.5% (rel.)). No other organ/tissue weights were significantly altered in the DINP or DINP + DEHP treatment groups for either sex. In the DINP + DEHP + DBP treatment group, male and female absolute pancreas weight increased (29.6 to 38.8%), female absolute (75%) and relative (50%) gonadal fat weight increased, female absolute spleen weight increased (34.8%), and male and female relative brain weight decreased 10-15%. Treatment with DINP caused a trend ($p=0.10$) in change in coat color distribution in $A^{vy}$/a offspring, whereas treatment with DINP + DEHP or DINP + DEHP + DBP significantly altered coat color distributions in $A^{vy}$/a offspring. Methylation status at four CpG sites in the promoter of the $A^{vy}$/a allele was determined to corroborate changes in coat color distribution. The high-methylation group females treated with DINP + DEHP + DBP has a slight increase in $A^{vy}$/a DNA methylation (60% vs. 50%) compared to controls. Compared to controls, no significant differences were observed in methylation status of male pups or among the pups categorized as low-methylation. Results for the DINP and DINP + DEHP high-methylation category was not reported. Global intracisternal A-particle (IAP) DNA methylation was next determined. Treatment with DINP caused a trend ($p=0.09$) in increased global methylation in female (but not male) $A^{vy}$/a offspring, while treatment with DEHP + DINP caused a significant increase in global methylation in both female and male $A^{vy}$/a offspring. Collectively, these results led study authors to conclude that gestational exposure to certain phthalates can influence body weight and organ weight early in life, and that these changes are associated with altered DNA methylation at IAPs.

i. Effects of Diisononyl Phthalate on Osteopenia in Intact Mice (Hwang et al. 2017)

Osteopenia is characterized by bone loss and deterioration of bone structure leading to fractures. Osteopenia is prevalent in industrialized areas and is associated with exposure to endocrine disrupting chemicals (EDCs). DINP is one of these EDCs extensively used in flexible polyvinyl chloride (PVC) products. Although it is well known that exposure to DINP

is harmful to humans, no studies have been reported concerning its contribution to osteopenia. In a study to elucidate the endocrine disrupting function of phthalates, DINP was administered to eight-week-old female C3H/HeN OVX mice (5 animals/group); a sham-operated control group, which were injected with PBS i.p.; a vehicle treated OVX group, which were injected with PBS, i.p.; and three DINP groups of 2, 20 or 200 mg of DINP/kg BW (body weight) daily (i.p.), respectively. The vehicle and DINP were administered for 6 weeks, and the body weights were recorded weekly. At the end of the 6-week treatment period, the animals were sacrificed by cervical dislocation. Sera were collected and stored at −80 °C until use, and the uteruses, tibias and femurs were removed and weighed. The femur and tibia lengths were measured which are indicators for osteopenia (bone loss and deterioration of trabecular bone). The following were measured from the collected blood samples calcium (Ca), inorganic phosphorus (IP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total cholesterol (TCHO), tartrate-resistant acid phosphatase (TRAP) activity (a marker of bone resorption) and serum estradiol (E2) levels, bone morphometric parameters of the distal femora cleaned of adherent soft tissues, including the tissue volume (TV), bone volume (BV), bone volume/tissue volume (BV/TV), bone surface (BS), bone surface/tissue volume (BS/TV), and trabecular thickness/separation/number (Tb.Th, Tb.Sp, and Tb.N) along with histological analysis of the distal femoral bone.

DINP exposure and OVX contributed to a significant increase to the final body weight six weeks after OVX surgery of C3H/HeN mice ($p < 0.001$) suggesting that indicate that DINP has effect on the body weight of the normal mice. DINP-exposed mice were found to be markedly reduced in uterus weight ($p < 0.001$) as well as tibia and femur length compared to those of the SHAM group in all the doses tested. Although, OVX increases the IP release compared to SHAM group, DINP treatment of the normal mice increased the IP release, however at the end of the experiment (6 weeks), there is no significant difference between the SHAM group and OVX group. DINP exposure at 20 mg/kg lowered the ALP activities than the SHAM group but the ALP activity in the DINP 200 mg/kg group did not increase significantly, though it increased numerically. LDH remain unchanged due to DINP exposure suggesting that DINP affects some biochemical markers in normal mice. Furthermore, the tartrate-resistant acid phosphatase (TRAP) activity (bone resorption marker) was increased, and the bone alkaline phosphatase (BALP) activity was lowered by the treatment with DINP

as compared with the SHAM group. Further, the microarchitecture of the femur and tibia in the intact mice was destroyed by the DINP injection. The tissue volume (TV), bone volume (BV), BV/TV, bone surface (BS), BS/TV, trabecular thickness (Tb.Th), and trabecular number (Tb.N) were reduced and the trabecular pattern factor (Tb.Pf), structure model index (SMI), and trabecular separation (Tb.Sp) were increased by the DINP injection. The bone mineral density (BMD) of the femur and tibia was lower in the DINP group than in the SHAM group. These results indicate that DINP contributes to an increased risk of osteopenia via destruction of the microarchitecture and enhancement of osteoclast activity, although it is difficult to conclude whether DINP has antiosteogenic activity or osteoclastogenic activity on the bone metabolism.

c) Diisononyl Phthalate Aggravates Allergic Dermatitis by Activation of NF-kB- (Kang et al. 2016)

To investigate the possible link between exposure to DINP and the development of allergies. 5–6-week-old male Balb/c mice were randomly divided into 8 groups (6 mice/group) and were gavaged with saline or DINP (2, 20 and 200 mg/(kg/d) from day 1 to 21, then sensitized with 120 μl of saline or 0.5% FITC (in 1:1acetone/DBP) on days 22 and 23, on their shaved backs. On day 28, the mice were challenged with 20 ul of saline or 0.5% FITC to the right ear, and saline or vehicle (1:1 DBP/acetone) to the left ear and the baseline ear thickness was measured. The experiment was terminated on day 29, and the blood samples were collected to determine, the IgE levels and immunohistochemistry was performed on the sections from the right ear for TSLP, p-STAT3, p-STAT5, p-STAT6, NF-κB and p65. Oxidative biomarkers such as ROS, MDA and GSH along with IL-4 and IFN-γ were measured from the ear tissue. One day after the final challenge ear edemas and an increase in ear weight were found in the FITC-immunized groups with no significant changes to the mice exposed to DINP or the saline group. However, exposure to DINP with increasing exposure was shown to aggravate ear edema and to significantly increase ear weight when compared with the group exposed to FITC only. Interestingly, treatment with PDTC, a well-known inhibitor of NF-κB, the ear swelling was markedly reduced; FITC+DINP200+PDTC group was compared with the FITC+DINP200 group ($p<0.05$). The bilateral ear weight decreased significantly ($p<0.05$) when the FITC+DINP-immunized groups were treated with pyrollidine dithiocarbamate (PDTC). DINP exposure alone showed no significant

pathological changes, FITC only group showed inflammatory cell infiltration into the skin, combined administration of DINP and FITC saw an increase in the number of infiltrating inflammatory cells. The pathological adverse effects seen in higher dose groups were alleviated with PTDC treatment, suggesting that exposure to DINP alone does not cause allergic dermatitis, but exposure could exacerbate the allergic dermatitis effects induced by FITC.

This deterioration was concomitant with increased total serum immunoglobulin-E and Th2 cytokines in the high dose groups (200 mg/day) along with FITC. Mice treated with PDTC showed a significant decrease in T-IgE levels ($p<0.05$). DINP exposure induced increase in ROS and MDA levels, and a decrease in GSH levels compared to saline treated group ($p<0.01$). Kang et al. (2016) determined the oxidative damage and the activation of nuclear factor-kb (NF-kB). The data demonstrated that DINP could promote oxidative damage and the activation of NF-kB in the skin. The expression of thymic stromal lymphopoietin and the activation of signal transducer and activator of transcriptions 3, 5, and 6 were enhanced concomitant with exacerbated allergic dermatitis effects and the activation of NF-kB induced by DINP.

d) Long-Term Dermal Exposure to Diisononyl Phthalate Exacerbates Atopic Dermatitis through Oxidative Stress in an FITC-Induced Mouse Model (Wu et al. 2015)

To elucidate the effect of DINP on allergic dermatitis (AD) in an FITC-induced allergic dermatitis model and the role of oxidative stress and inflammatory factors in skin lesions of the model mice and characterize the mechanism involved in the DINP induced deleterious effect. Additionally, the effects of melatonin (MT) on the inhibition of AD and explore its mechanism as an antioxidant. Male Balb/c mice were divided randomly into seven groups of 7 mice. Forty-nine male Balb/c mice were divided randomly into seven groups: (i) control group (control); (ii) melatonin (30mg/(kg/d)) 3 h after saline skin exposure (MT); (iii) 0.5% FITC sensitized group (FITC); (iv) 1.4 mg/ (kg/d) DINP skin exposure combined with 0.5% FITC sensitized group (FITC + DINP1.4); (v) 14.0 mg/(kg/ d) DINP skin exposure combined with 0.5% FITC sensitized group (FITC + DINP 14); (vi) 140.0 mg/(kg$ d) DINP skin exposure combined with 0.5% FITC sensitized group (FITC + DINP 140); (vii) MT (30mg/(kg$ d)) 3 h after 140.0 mg/(kg/d) DINP skin exposure combined with 0.5% FITC sensitized group (FITC + DINP 140.0 + MT). The experiment was terminated on day 48, and

the blood samples were collected to determine, the IgE levels and immunohistochemistry was performed on the sections from the right ear for TSLP, p-STAT3, p-STAT5, p-STAT6, NF-κB and p65. Oxidative biomarkers such as ROS, MDA and GSH along with IL-4 and IFN-γ were measured from the ear tissue. High dose DINP with FITC (FITC + DINP 140) increased the number of infiltrating inflammatory cells when compared with the FITC group ($p<0.05$). Furthermore, the pathological alterations and the number of infiltrating inflammatory cells were alleviated in the FITC + DINP 140 + MT group as compared with the FITC + DINP 140 group. Ear swelling as well as in the bilateral ear weight were significantly ($p<0.01$). altered in FITC-immunized groups (FITC, FITC + DINP1.4, FITC + DINP14, FITC + DINP140, FITC + DINP140 + MT). DINP significantly aggravated ear swelling and bilateral ear weight when compared to the group exposed to FITC only, and this exacerbating effect was enhanced with increasing DINP exposure. However, MT attenuated DINP induced ear swelling and the bilateral ear weight (FITC + DINP 140 group with the FITC + DINP 140 + MT group ($p<0.01$). DINP synergistically enhanced serum T IgE levels, All FITC-sensitized groups showed an increase ($p<0.01$) in T-IgE concentration as compared with the control group. High doses of DINP (140 mg/kg) exposure significantly elevated the quantity of T-IgE ($p<0.05$) in the serum compared with the FITC-sensitization only group. As seen with preceding results, T-IgE levels in the FITC + DINP 140 group, compared to FITC + DINP 140 + MT group decreased significantly ($p<0.01$). To evaluate the association between dermal exposure to DINP and Th cytokine expression, Th1 cytokine (IFN-g) and Th2 cytokine (IL-4 and IL-5) concentrations and the ratio of IL-4 to IFN-g in the ear tissue were determined. Although, dermal exposure to MT alone did not have any effect on cytokine expression, they were significantly higher in FITC-immunized group ($p<0.01$) Compared with the FITC group, there was a significant increase of IL-4, IL-5 and a resulting skew in the ratio of IL-4 to IFN-g in the FITC + DINP groups, and with an increasing concentration of DINP, the exacerbation effect was stronger ($p<0.05$). MT alleviated the DINP effect ($p<0.01$), suggesting that DINP is associated with Th2 cytokine expression by FITC-mediated allergic inflammation. DINP via dermal exposure, aggravated oxidative stress in the swollen ear and the MT alleviated the stress. ROS levels in the 140 mg/kg DINP exposure group showed that there was an significant difference ($p<0.01$) when compared to the control group, and a significant difference ($p<0.05$) when compared to the FITC group. GSH levels, in the control group, compared to all the FITC-immunized groups (FITC, FITC +

DINP1.4, FITC + DINP14, FITC + DINP140, FITC + DINP140 + MT) showed significant changes ($p<0.01$) and MT alleviated the effects with ROS production and normalized the GSH levels. The results of a histopathological examination and measurement of ear swelling as well as immunological and inflammatory biomarkers (total-immunoglobulin (Ig)E and Th cytokines) supported the notion that high doses of DINP may aggravate atopic dermatitis. Wu et al. (2015) also showed that melatonin, an antioxidant, could decrease the levels of oxidative stress and alleviate FITC-induced CHS, suggesting that oxidative stress may be one of the molecular mechanisms to explain the exacerbation effect induced by DINP.

e) Determination of *in vivo* Estrogenic Potential of Di-isobutyl Phthalate (DIBP) and Di-Isononyl Phthalate (DINP) in Rats (Sedha et al. 2015)

To evaluate the estrogenic potential of phthalate esters DINP and DIBP, two different assays were employed: a 3-day uterotrophic assay and 20-day pubertal female assay. For 3-day uterotrophic assay, immature female rats (20 days old) were randomly divided into six different groups with a minimum of six animals in each group. The immature female rats were treated orally with two different doses of DIBP (250 and 1250 mg/kg), DINP (276 and 1380 mg/kg) in corn oil and a single dose of DES (40 µg/kg) in corn oil once daily for three consecutive days. Control and vehicle control (corn oil) groups were also maintained. Body weight and clinical signs and symptoms were recorded daily. The animals were sacrificed on day 4. The uterus and ovaries wet weight (mg) were recorded. In the 20-day pubertal female assay, immature female rats were treated orally with DIBP (250 and 1250 mg/kg), DINP (276 and 1380 mg/kg), and DES (6 µg/kg) in corn oil daily from post-natal day (PND) 21 for 20 days. Vaginal opening was checked/recorded daily from PND 21 till necropsy. Body weight of the animals was recorded before treatment and every day thereafter. Animals were sacrificed on PND 41. DINP and DIBP exposure did not significantly alter the body weight gain in the uterotrophic assay. The results indicate that non-significant alterations in uterine and ovarian wet weight were observed in DINP and DIBP-treated groups while the uterus weight increased significantly (i.e., 4–6 times) in the diethylstilbesterol (DES)-treated group (positive control) in both the assays. No precocious vaginal opening was found in any of the DINP and DIBP-treated groups compared to DES-treated group, which were found to have precocious vaginal opening occurred on day 26. These results indicate that DINP was unable to induce elevation in the uterine weight in both the assays and unable to cause vaginal

opening indicating non-estrogenic potential of both the phthalate compounds *in vivo*.

f) Mice Brain Tissue Injury Induced by DINP Exposure and the Protective Application of Vitamin E (Peng 2015)

The adverse effects of DINP exposure on mouse brain were evaluated by Morriswater maze (MWM) test and histological assay. To investigate the brain tissue injury induced by DINP exposure. Specified pathogen-free (SPF) class male Kunming mice (5–6 weeks old, 22 ± 2 g) were divided randomly into six experimental groups of 10 animals. These groups were exposed to 0, 1.5, 15, and 150 mg/kg/day DINP, 150 mg/kg/day DINP + 50 mg/kg/day vitamin E (block group), and 50 mg/kg/day vitamin E. DINP or vitamin E was administered for 9 days. During this period, MWM was used to investigate the cognitive ability of the mice and mice were euthanized at 10th day, 24 h later after the last DINP exposure, mouse brains were collected for quantifying the indices of oxidative stress (reactive oxygen species (ROS), malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) activity), inflammation (tumor necrosis factor alpha (TNF-$\alpha$), interleukin-1 (IL-1)), and apoptosis (caspase-3 and caspase-3 activity) to explore the possible mechanisms. There was no significant change in body weight of the mice treated with DINP compared to the control group. There was no significant change in each group in the brain index change of mice after DINP exposure. A reduction in escape latency was observed in all groups of mice after DINP exposure, throughout 7 days training. The latency of the control group had the fastest decrease, whereas the 150 mg/kg/day group had the slowest decrease. A significant increase ($P < 0.05$) in average escape latency for 7 days was detected in the mice from the 150 mg/kg/day groups compared with the mice from the control group, however, after vitamin E protection, compared with the mice in 150 mg/kg/day group, the mice in the block group spent shorter times ($P < 0.05$) finding the platform. Spatial memories of the mice were evaluated after the forgetting period (the 8th day). The time of each mouse remaining in the target quadrant (SE quadrant) was different, mice in the 150 mg/kg/day groups were significantly decreased ($P < 0.05$), the mice in blocked group spent longer times in the target quadrant compared with the mice in the 150 mg/kg/day groups ($P < 0.05$). The routes taken by the mice in the control and blocked groups were purposeful, orderly, and focused on the target quadrant (SE), while the routes taken by the mice in 150 mg/kg/day groups were irregular and apparently without purpose, suggesting that the cognitive abilities of the mice

were affected by the DINP exposure. Oxidative stress biomarkers in the highest dose group tested (150 mg/day) showed a significant increase in ROS level ($P < 0.05$), a significant reduction ($P < 0.01$) in GSH content, an increase in the MDA content ($P < 0.01$) and a reduction in SOD activity levels ($P < 0.05$). After vitamin E protection, compared with the mice in 150 mg/kg/day group, ROS and MDA levels of mice in blocked group were decreased ($P < 0.01$, $P < 0.05$), and the level of SOD was increased ($P < 0.05$). However, after vitamin E protection, the level of GSH in block group mice was not changed. DINP-exposed mice brain showed oxidative stress, inflammatory responses, apoptosis, and pathological alterations in the hippocampus. Additionally, these exacerbations were counteracted by vitamin E. Oral exposure of mice to DINP induced brain damage, and oxidative stress, inflammation, and the consequential apoptosis jointly constituted the potential mechanisms of such induced toxicity.

g) Cognitive Deficits and Anxiety Induced by Diisononyl Phthalate in Mice and the Neuroprotective Effects of Melatonin (Ma et al. 2015)

Diisononyl phthalate (DINP) is a plasticizer frequently substituted for other plasticizers that are prohibited in consumer products. *In vivo* studies on the neurotoxicity of DINP are however, limited. To investigate whether DINP causes neurobehavioral changes in mice, Forty-nine Kunming mice (5–6 weeks old, 22 ± 2 g) were randomly allocated were randomly assigned to one of seven groups (7 mice/group): vehicle control (0.9% NaCl/Tween 80 1:1), 0.2, 2, 20, 200 mg/kg/day (DINP/Tween 80 1:1), 50 mg/kg/day Mel (the melatonin solution was 5 mg/mL), 200 mg kg/day DINP+ 50 mg/kg/day Mel, in a dosing volume of 10 mL/kg body weight. Animals were gavage fed for 14 consecutive days, from the $6^{th}$ to $12^{th}$ day the animals performed the hidden-platform acquisition test, by Morriswater maze (MWM), on the 13th day they were not subjected to any activity (the forgetting-period), and on the 14th day they were given the probe trial test and open-field test (OFT). 24 hrs after the final dose, the animals were sacrificed and the brain tissue was collected for histological examination and the brain tissue was prepared for detection of oxidative stress (ROS, GSH, SOD, 8-OH-dG assay, DNA protein cross links (DPC) and inflammation (TNF-α and IL-1β).

MWM is a behavioral test that is designed to evaluate the cognitive abilities of animals. after 7 days (from the 6th to 12th day) of training the escape latency was reduced in each group. The escape latency for the control group mice showed the greatest decrease over the 7

days period, whereas that for the 200 mg/kg/day group showed the least decrease. A significant increase ($P < 0.05$) in the average escape latency for 7days was detected in the mice from the 200 mg/kg/day group, compared with the mice from the control group. The mice were not disturbed on 13$^{th}$ day (the forgetting-period), and then the spatial memory ability of the mice after DINP exposure was evaluated on the 14th day. The time spent by the mice in the 200 mg/kg/day group was significantly decreased ($P < 0.01$), and the frequency of entry (times of one test) into the target quarter showed the same trend ($P < 0.05$). From the swimming pathway data from the 14th day, it is clear that the mice in the control groups swam purposefully, and were focused on the target, whereas the pathways of the mice in the 200 mg/kg/day group were irregular and showed little purpose attuned to learning disability. DINP exposure causes anxiety, The OFT used to assess the anxiety-like emotions of animals, there was significant decreases in central/total area distance ($P < 0.05$) and central/total area entries ($P < 0.01$) in the 200 mg/kg/day group when compared to the controls and the animals were concentrated in the border area. The defecation number also showed significant increases ($P < 0.01$) in the 20 and 200 mg/kg/day groups indicating stress. Pathological changes were observed in the brains of mice exposed to increasing concentrations of DINP, ranging from loose and disordered arrangements of cells through swelling deformations of cells to the shortening or even to the disappearance of apical dendrites. Immunohistochemical analysis showed that 20 or 200 mg/kg/day DINP exposure groups showed significantly enhanced levels ofcaspase-3 and GFAP both in the CA1 region of the hippocampus and in the cerebral cortex. DINP treatment increased the oxidative stress level in brain tissue of 20 and 200 mg/kg/day groups, significant increases ($P < 0.05$) in ROS levels, significant decrease in GSH ($P < 0.05$) and SOD activity ($P < 0.05$. DPC and 8-OH-dG were used to measure the DNA damage associated with oxidative stress, both 8-OH-dG (20 and 200 mg/kg/day groups) and DPC (200 mg/kg/day group) increased significantly ($P < 0.01$) after DINP exposure. DINP exposure induced inflammation in brains. TNF-α and IL-1β in expression of the 20 and 200 mg/kg/day groups increased significantly ($P < 0.01$). Melatonin alleviated DINP induced effects, ROS generated in the DINP+ Mel group was significantly less ($P < 0.05$) than that in the 200 mg/kg/day DINP alone group. GSH levels in the DINP+ Mel group were significantly higher ($P < 0.05$). The activities of SOD showed a similar trend to GSH, with the activities of the DINP+ Mel group increasing significantly ($P < 0.01$). The damage to DNA was also mitigated by 8-OH-dG and

DPC in the DINP+ Mel group, which decreased significantly compared with that of the 200 mg/kg/day DINP alone group ($P < 0.01$ and $P < 0.05$). The release of TNF-α and IL-1β in the DINP+ Mel group also decreased compared with the 200 mg/kg/day DINP alone group. DINP exposure in high concentrations (20 or 200 mg/ kg/day) decreased the cognitive abilities and induced anxiety in mice. DINP exposures results in oxidative stress that caused damage to the mouse brain. Melatonin offered protection and decreased oxidative stress levels.

h) Effects of Oral Administration of DEHP and DINP on Atopic Dermatitis in NC/Nga Mice (Sadakane et al. 2014)

Phthalates are known to cause adjuvant like effects on immunoglobulin production. DEHP and DINP in low doses have been shown to cause aggravation of atopic dermatitis-like skin lesions (ADSLs) in mouse models. NC/Nga mouse model were used to investigate the effects of oral administration of DINP at doses lower than the NOAEL on atopic dermatitis (AD) in mice. 120 male NC/Nga mice aged 7 weeks (22-25 g) were used out of which 60 mice each were used to investigate the effect of DEHP and DINP on AD and. In each of the 2 experimental conditions, 12 mice each were placed into 1 of 5 groups, the saline vehicle group (the control group) or 1 of the 4 experimental groups. Animals in the experimental groups were exposed to the allergen by subcutaneous injection of 5 mg of *Dermatophagoides pteronyssinus* (Dp) dissolved in 10 ml of saline in the ventral side of the right ear 2–3 days a week (a total of 8 times) under anesthesia. Animals in the experimental groups for DEHP exposure were exposed to the allergen and treated with 0 (Dp+vehicle), 8.3 (Dp+DEHP 8.3), 166.3 (Dp+DEHP 166.3) or 3325 (Dp+DEHP 3325) mg/animal, and animals in the DINP groups were exposed to the allergen and treated with 0 (Dp+vehicle), 6.6 (Dp+DINP 6.6), 131.3 (Dp+DINP 131.3) or 2625 (Dp+DINP 2625) mg/animal. Once a week for four weeks, the experimental groups were orally administered the appropriate dose of DEHP or DINP dissolved in 0.1mL of olive oil either three (DEHP groups) or five (DINP groups) days before the first injection of the allergen. Animals in the saline + vehicle groups and Dp+vehicle groups were not exposed to DEHP or DINP and were orally administered 0.1mL of olive oil only. Twenty-four hours after each subcutaneous injection, ear thickness was measured and scored for symptom of skin dryness and eruption, edema, crusting and erosion. Skin disease symptomatology was subsequently evaluated, Dp-treated groups had markedly higher clinical

scores including those for dryness, redness, erosion, crust formation, edema and ear thickening, than the control groups. Although the clinical scores of the Dp+DEHP 3325 group increased significantly by day 8 ($p<0.01$) and those of the Dp+DEHP 166.3 group by day 11 ($p<0.05$). Clinical scores of the Dp+DINP 131.3 and Dp+DINP 6.6 groups began increasing compared with the Dp+vehicle group from day 16, the Dp+DINP 131.3 group had a higher (not significant) wound score compared with the Dp+vehicle group while the Dp+DINP 2625 did not change. ANOVA and Dunnett's multiple comparison test revealed no significant differences between either DEHP or DINP treated groups to control at any doses to contribute to ASDLs. The eosinophil count in the subcutaneous tissue of the DP treated group increased significantly ($p<0.05$). Except for that of the Dp+DEHP 3325 group, which was significantly higher than that of the Dp+vehicle group ($p<0.05$), the eosinophil count of the DEHP-exposure groups had increased but not significantly. The number of severely degranulated mast cells and total number of mast cells in the was also higher ($p<0.05$). The dorsal skin of the Dp-treated groups with or without DINP exposure exhibited epidermal and dermal thickening, eosinophil accumulation and mast cell degranulation. The eosinophil counts of both DP+DINP treatments increased but not significant. Oral exposure to DEHP tended to increase the IL-13 level of all the groups, particularly that of the Dp+DEHP 166.3 group, which was statistically greater than that of the Dp+vehicle group ($p<0.01$). However, oral exposure to DINP did not increase the eotaxin levels. Mite-allergen significantly increased total serum IgE ($p<0.01$) and Dp-specific IgG1 levels. While oral exposure to DEHP did not increase Ig production, exposure to DINP modestly increased mean total IgE levels. The contribution of a given dose of DEHP or DINP to the results of pathological analysis and ELISA were evaluated by the Spearman's rank correlation coefficient. For DEHP, the ranking of the mean skin scores in the DEHP-exposure experiments (Dp+Vehicle >Dp+DEHP 166.34 >Dp+DEHP 8.34 >Dp+DEHP 3325) was found to be weakly positively correlated with the level of total IgE ($p=0.339$, $p<0.05$) and moderately negatively correlated with the number of the eosinophils ($p=0.471$, p50.05). For DINP, the ranking of the mean skin scores in the DINP-exposure experiments (Dp+DINP 131.3 >Dp+DINP 6.64 >Dp+DINP 26254 >Dp+vehicle) was found to be strongly positively correlated with the number of eosinophils ($p=0.651$, $p<0.01$) and the number of severely degranulated mast cells ($p=0.706$, $p<0.001$), and moderately positively correlated with the total number of mast cells ($p=0.541$, $p<0.01$). DINP administration tended to aggravate allergen-induced ADSL production. Oral

administration of both DEHP and DINP at doses lower than the NOAEL tends to increase the allergic response in animal AD models, but only DINP administration slightly aggravates allergen-induced ADSL production.

i) Effects of Diisononyl Phthalate on Atopic Dermatitis *in vivo* and Immunologic Responses *in vitro* (Koike et al. 2010)

Diisononyl phthalate (DINP), has been shown to have an adjuvant effect on immunoglobulin (Ig) production. However, the effects of DINP on allergic diseases have not been fully elucidated. To investigate the effects of DINP on atopic dermatitis (AD)-like skin lesions induced by Dermatophagoides pteronyssinus (Dp) in atopic-prone NC/Nga mice in vivo and on the immunologic responses of BMDCs and splenocytes in vitro. Six-week-old SPF NC/NgaTndCrlj male mice were divided into six groups and were injected intradermally on the ventral side of their right ears with saline or 5 μg mite extract *Dermatophagoides pteronyssinus* (Dp), dissolved in 10 μL saline on study days 0, 3, 5, 8, 10, 12, 15, and 17 under anesthesia. DINP at a dose of 0, 0.15, 1.5, 15, or 150 mg/kg/day dissolved in 0.1 mL olive oil (vehicle), was injected intraperitoneally (IP) on days –5, 2, 9, and 16 from the first Dp treatment. Twenty-four hours after each Dp injection, ear thickness and clinical scores were evaluated. Clinical scores, ear thickening, histologic findings, protein expression of cytokines/chemokines in the ear, and serum levels of Immunoglobulins (Ig) and histamine were measured. Additionally, the effects of DINP on bone-marrow-derived dendritic cells (BMDCs) or splenocytes *in vitro* were also measured. DINP affects AD-like skin lesions induced by Dp, ear thickening and observed macroscopic features were significantly enhanced compared with saline injection from 4 days after the first injection of Dp ($p < 0.05$). DINP exposure via IP significantly enhanced ear thickening compared with vehicle in the presence of intradermal Dp from 6 days after the first injection of Dp ($p < 0.05$). However, there was no dose-dependent effects of DINP. The ear thickening was most prominent in animals treated with 15 mg/kg/day DINP in the presence of intradermal Dp ($p < 0.05$). Dp induced the infiltration of eosinophils into the skin lesions compared with saline ($p < 0.05$). DINP (15 mg/kg/day) aggravated the infiltration of eosinophils into the skin lesion compared with vehicle in the presence of intradermal Dp, paralleled to the severity of mast cell degranulation. Though, Dp significantly increased the expression of IL-4, IL-5, and IL-13 and significantly decreased the expression of IFN-γ compared with saline injection. DINP did not

affect the expression of these cytokines, although DINP decreased IFN-γ expression compared with vehicle. Dp increased the expression of eotaxin, eotaxin-2, and TSLP compared with controls (eotaxin, $p < 0.01$). However, DINP significantly decreased the expression of eotaxin and eotaxin-2 compared with vehicle in the presence of intradermal Dp ($p < 0.05$). On the other hand, DINP at 0.15 mg/kg/day significantly increased TSLP expression compared with vehicle in the presence of intradermal Dp ($p < 0.05$). Dp significantly increased the levels of Dp-specific IgG1 and total IgE and tended to increase histamine levels in serum compared to controls ($p < 0.01$]. DINP treatment failed to affect the levels of Dp-specific IgG1 and total IgE in serum. In the presence of Dp, DINP significantly increased histamine levels in serum ($p < 0.01$). DINP exposure for 24 hr significantly increased the production of TH2 chemokines, TARC/CCL17 (100 μM DINP, $p < 0.05$) and MDC/CCL22 (100 μM DINP, $p < 0.05$), from BMDCs compared with control (0 μM DINP). However, TH1 cytokine IL-12p40 in any BMDC cultures were not detected. DINP also significantly increased the expression of the chemokine receptors CCR7 (100 μM DINP, $p < 0.01$) and CXCR4 (10 or 100 μM DINP, $p < 0.05$), MHC class II (1 or 10 μM DINP, $p < 0.01$; 0.1 or 100 μM DINP, $p < 0.05$), CD80 (100 μM DINP, $p < 0.05$), and CD86 (1, 10, or 100 μM DINP, $p < 0.01$) on BMDCs compared with controls. DINP exposure for 24 hr significantly increased IL-4 production from splenocytes compared with controls (10 μM DINP, $p < 0.05$; 100 μM DINP, $p < 0.01$). A 72-hr exposure to DINP in the presence of Dp significantly increased proliferation of splenocytes at 0.001–1 μM and decreased the proliferation at 10 μM compared with controls (0.001 or 1 μM DINP, $p < 0.01$; 0.01, 0.1, or 10 μM DINP, $p < 0.05$). DINP aggravated AD-like skin lesions related to Dp. The aggravation was consistent with eosinophilic inflammation, mast cell degranulation, and thymic stromal lymphopoietin (TSLP) expression in the ear. DINP enhanced the expression of cell surface activation markers on BMDCs and their production of TARC/CCL17 (thymus- and activation-regulated chemokine) and MDC/CCL22 (macrophage-derived chemokine), as well as their capacity to stimulate Dp-specific T-cell proliferation. DINP also enhanced interleukin-4 production and Dp-stimulated proliferation of splenocytes to conclude that DINP can aggravate AD-like skin lesions related to Dp. The mechanism of the aggravation was suggested to be mediated, at least partly, through the TSLP-related activation of dendritic cells and by direct or indirect activation of the immune cells.

j) Effects of Phthalate Esters on the Sensitization Phase of Contact Hypersensitivity Induced by Fluorescein Isothiocyanate (Imai et al. 2006)

DBP had an adjuvant effect during the sensitization phase with fluorescein isothiocyanate (FITC) in a mouse contact hypersensitivity model involving FITC as a hapten. Phthalates such as DBP facilitates the trafficking of FITC-presenting cells to the draining lymph nodes from skin sites where the hapten had been applied. The facilitated trafficking of FITC-presenting cells was shown to be one of the factors that influences the extent of sensitization. To investigate if different phthalate esters have adjuvant effects on skin sensitization with FITC and are correlated with the effect on the trafficking of FITC-presenting dendritic cells or macrophages from the skin to draining lymph nodes. Specific pathogen-free female CD-1 (ICR) and BALB/c mice were used for sensitization; acetone, acetone (A)/DMP (A/DMP) (1 : 1, v/v), A/DEP (1 : 1), A/DPP (1 : 1), A/DBP (1 : 1), A/DEHP (1 : 1), and A/DINP (1 : 1), and the applications were repeated on day 7 and 14. Ear thickness and ear swellings were measured. Five-week-old ICR mice were epicutaneously sensitized with FITC dissolved in acetone containing one of various phthalate esters. Considerable variation between individual mice in the ear-swelling response, the groups sensitized in the presence of DBP, DPP, and DMP exhibited significantly stronger responses at 24 h as compared with the control group that had been sensitized with FITC in acetone. DEHP and DINP, the levels of the responses were not statistically different from that in the control group. A similar results were confirmed using an (8-week old) inbred mouse strain, BALB/c. Draining lymph node cells obtained 24 hours after skin sensitization were examined for FITC fluorescence by means of flow cytometry. FITC-positive cells were characterized with anti-CD11c and anti-CD11b through three-color flow cytometry. Mice sensitized with FITC in acetone containing DINP did not show consistent ear-swelling response as seen with DBP. Upon sensitization in the presence of DBP, the number of FITC-positive dendritic cells (total CD11c+ as well as CD11c+/CD11b+) was increased in draining lymph nodes. DINP showed no significant increase in the FITC-positive cell number in the draining lymph nodes.

k) DEHP and DINP Induce Tissue- and Gender-Specific Disturbances in Fatty Acid and Lipidomic Profiles in Neonatal Mice: A Comparative Study (Huang et al. 2019).

Transcriptomics and nontargeted global metabolomics both demonstrated the alteration of lipid metabolism as a consequence of DEHP exposure. DEHP is being replaced with DINP

in most used products. To explore differential effects of DEHP and DINP on (1) the distributions of FA compositions in different tissues and genders and (2) gender-specific changes of lipidomic profiles in plasma. Huang et al. (2019) investigated the potential effects of DINP and DEHP on lipid metabolism in mice. Six male and 18 female-specific pathogen-free Kunming mice (8−10 weeks old) were used for breeding (1male :2 females), the Dams were allowed to give birth naturally at term. Newborn mice (PND0) remained with their natural mothers in the same cage until PND21. Each cage was randomly assigned to a treatment (DEHP or DINP) or control group. Each group contained three replicate cages. Both male and female pups were exposed to vehicle control (corn oil), DEHP (high dose 4.8 mg/kg bw/ day or low dose 0.048 mg/kg bw/day), or DINP (high dose 4.8 mg/kg bw/day or low dose 0.048 mg/kg bw/day) through subcutaneous injection daily from PND0 to PND21. At PND22, 10 male and 10 female pups were randomly selected from three replicate cages per treatment group. These pups were selected for phthalate metabolite residue, biochemical, and FA/lipidomic analyses. Phenotypic and biochemical information from mice exposed to Control, DEHP, and DINP at two doses (4.8 or 0.048 mg/kg bw/d) at PND22 were collected. Mice exposed to the low and high doses of DEHP exhibited urinary MEHP concentrations of 2.9 ± 0.6 and 123 ± 133 ng/mL at PND22, respectively, whereas urinary concentrations were below LOQ in control groups. In addition to MEHP, DEHP's secondary metabolites, including MECPP, MEOHP, and MEHHP, were also detected with a combined concentration of 69.7 ± 23.6 and 3426 ± 1140 ng/mL in mice exposed to the low and high doses of DEHP, respectively. In DINP-treated mice, concentrations of urinary MINP (a major metabolite of DINP) and MCOP (secondary metabolite) were detected to be 36.4 ± 5.4 and 3570 ± 196 ng/mL in the low- and high dose groups, respectively. No significant differences were observed in the concentrations of any metabolites between genders. Neonatal exposure to DEHP or DINP altered number of phenotypes in mice, while the impacts differed between chemicals and dosages. At PND22, both male and female pups exposed to the high dose of DEHP exhibited lower body weights compared with control groups, although the body mass index (BMI) was not changed. By contrast, exposure to the high dose of DINP did not significantly change body weights or BMI of pups. High dose of DEHP decreased kidney, heart, and brain weights exclusively in the males, while the high dose of DINP treatments diminished male heart weight compared to the control pups. Biochemical assays showed

inconsistent trends of changes in plasma total cholesterol, total TGs, diglycerides, and liver TBA in male and female pups. High dose of DEHP and DINP could cause major alterations in FA compositions (i.e., more than 12 out of 33 FAs affected) in plasma, white adipose tissue, and heart and some lesser alterations (i.e., 3−11 out of 33 FAs affected) in liver, whereas no significant changes were observed in kidney and brain. High dose of DEHP significantly decreased SFA composition but increased the PUFA content in female pups, while it significantly decreased the PUFA content and increased the Trans level in males. By contrast, high dose of DINP did not change FA compositions in female pups with the exception for Trans but significantly altered the compositions of SFAs, PUFAs, and Trans in male pups, suggesting that, male mice could be more effectively impacted by DINP than females when exposed to a high dose, whereas both genders could be affected by DEHP with respect to FA compositions. DEHP and DINP-treated animals differed in the patterns of disruption. DEHP or DINP exposure caused gender-specific alterations of eight lipid classes in plasma as identified by lipidomic analyses. In the high dose group, DEHP induced decreases in total phosphatidylcholines and phosphatidylinositol (PI) in females and increases in phosphatidylethanolamines (PEs) and triglycerides in males. In contrast, 4.8 mg/kg-day DINP caused alterations of PEs, PIs, phosphatidylserines, and cholesterols in male, but not female pups. Although the most significant dysregulation of lipid metabolism was often observed in animals of the high dose group, lipid profiles were also disrupted in low dose animals and for some alterations the magnitude of the change was greater in the low dose group. These results indicate that DINP can disrupt lipid metabolism in a tissue- and gender-specific manner.

| **Table [ SEQ Table * ARABIC ]. Summary of Non-apical Endpoints after Administration of DINP** | | |
|---|---|---|
| **Reference** | **Description of the Study** | **Effected Organ/Tissue** |
| Huang et al. 2019 | DEHP and DINP induce tissue- and gender-specific disturbances in fatty acid and lipidomic profiles in neonatal mice | Fatty acid profiles in neonatal mice |
| Deng et al. 2019 | Exposure to DINP induced an increase in blood pressure through activation of the ACE/ AT1R axis and inhibition of NO production | Systemic effects, increased blood pressure |
| Neier et al. 2019 | Perinatal exposures to phthalates and intracisternal A-particle (IAP) DNA methylation in weanling mice | DNA methylation and changes in sex specific effects |
| Hwang et al. 2017 | Effects of diisononyl phthalate on osteopenia in intact mice | DINP contributes osteopenia by the destruction of the microarchitecture and enhancement of osteoclast activity |
| Kang et al. 2016 | Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB- | DINP aggravates allergic-dermatitis-like lesions |
| Wu et al. 2015 | Long-term dermal exposure to DINP exacerbates atopic dermatitis through oxidative stress | high doses of DINP may aggravate atopic dermatitis |
| Sedha et al. 2015 | *In vivo* estrogenic potential of DINP in rats | DINP is unable to induce elevation in the uterine weight and unable to cause vaginal opening indicating non-estrogenic potential of DINP |
| Peng 2015 | Mice brain tissue injury induced by DINP exposure and the protective application of vitamin e | DINP induced brain damage, and oxidative stress, inflammation, and apoptosis |
| Ma et al. 2015 | Cognitive deficits and anxiety induced by DINP in mice | Histopathological alterations in the brain and increased levels of oxidative stress, and inflammation |
| Sadakane et al. 2014 | DINP on atopic dermatitis in NC/Nga mice | DINP at doses lower than the NOAEL tends to increase the allergic response in animal AD models, but only DINP administration slightly aggravates allergen-induced ADSL production |
| Koike et al. 2010 | DINP on atopic dermatitis *in vivo* and immunologic responses *in vitro* | DINP aggravated AD-like skin lesions related to Dp<br>DINP enhanced the expression of cell surface activation markers and interleukin-4 production |

| | | |
|---|---|---|
| Imai et al. 2006 | Effects of phthalate esters on the sensitization phase of contact hypersensitivity induced by fluorescein isothiocyanate | DINP showed no significant increase in the FITC-positive cell number |

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## Chapter 3
## Ecological Hazard Assessment

## I. Executive Summary

This technical report has been updated to reflect the currently available literature pertaining to the ecological effects of diisononyl phthalate (DINP) which is determined to be relevant to the assessment (see USEPA 2005). The Agency considered the information submitted in response to comments and conducted a literature search to determine if any additional published data wereavailable. This included new searches of the databases that are a routine part of the chemical review process: ECOTOX (2001), the Hazardous Substances Database (TOXNET), EPI Suite™ v4.11, EFED (2000) Pesticide Ecotoxicity Database, Science Direct, and SETAC *(Environmental Toxicology and Chemistry* Jan. 2005-Jan. 2022).

It should be noted that most of the studies this assessment was based on were conducted over 20 years ago, and the criteria at that time were considered acceptable by the Agency due to the available analytical methodology. Studies have been reevaluated using current criteria and it was determined that there were issues related to the water solubility of the chemical and therefore the ability to accurately determine the true toxicity to aquatic organisms. However, the Agency continued to allow these studies to satisfy the data requirements in order to evaluate the hazard of this chemical. If these studies were to be conducted again, the testing facility would be required to ensure the chemical was properly in solution when exposing the test species. EPA recognizes that most of the studies evaluated were tested at toxicant concentrations above the water solubility level but nevertheless considers these studies to be acceptable to assess environmental hazard.

Table 1 lists the experimental data that were considered scientifically acceptable[1] for assessing the environmental hazard of DINP. The test concentrations reported in these studies exceed (by as much as 3 orders of magnitude) current estimates of the solubility limit for DINP [approximately 0.6 µg/L (0.0006 mg/L)]. Assuming DINP was available in solution at approximate concentrations of 0.6 µg/L, no adverse effects were observed in 16 aquatic studies. The *Daphnia magna* life cycle study indicated effects on survival and reproduction at levels of

1. Studies were evaluated based on the criteria outlined in the Standard Evaluation Procedures EPA 540/8-85 series and EPA 540/9-86 series established by Office of Pesticide Programs and the Office of Prevention, Pesticides and Toxic Substances Testing Guidelines (850.1010 through 850.6200).

DINP of 0.089 and 0.17 mg/L, but this may be primarily attributed to the physical properties of DINP (a tendency to form a layer of chemical on the surface of the water) rather than a true toxic effect (Staples et al. 2011). EPA Ecological Structure Activity Relationships (ECOSAR) Predictive Model Application 2.0 results for modeled environmental toxicity data are presented in Attachments F-I (https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model).

Based on the available information, the Agency concludes that DINP will not cause adverse effects to the environment at levels below its water solubility limit. However, since phthalates may cause a concern for developmental effects to terrestrial species, the Agency may deem it appropriate at a later date to consider other possible ecological effects to species that have not been identified at the time of this report.

## II. Acute Invertebrate Toxicity

A. Freshwater

Springborn Bionomics (1984a) studied the acute toxicity of 14 phthalate esters to *Daphnia magna* under static conditions. Based on the 0 and 48 hour mean measured concentrations, the LC50 is >0.086 mg/L (CASRN: 28553-12-0). There was no visible film or apparent insoluble test material in the test solution; however, since there were entrapped daphnids on the test vessel's surface, it is suggested that the test material was present on the surface during the initial tests. Reported test conditions were appropriate for the type of study, with pH range of 7.9 - 8.3, a temperature of 22° C, and dissolved oxygen (DO) of 5.3 mg/L (60 % saturation). The measured DO was at the low end of the acceptable range but is probably attributable to the static conditions. Fifteen test organisms (5 per replicate beaker) were exposed to each phthalate ester concentration. Mortalities were measured at 0, 24, and 48 hours after test initiation. The concentrations for DINP ranged from 0.084 - 0.088 mg/L at test initiation to 0.037 - 0.035 mg/L at test termination. The nominal concentration of DINP was 20µl/L. No mortality was reported. However, more than 50% of the daphnids were caught on the surface of the test solution.

EG and G Bionomics (1984a) studied the acute toxicity of 12 phthalate esters, including DINP, to the midge *Paratanytarsus parthenogenica.* Since DINP has low water solubility, corroborative tests consisting of exposure of midge larvae to a single replicated concentration was used for measuring acute toxicity. The LC50 for this species is greater than 0.12 mg/L, and there was no effect observed at this concentration. Test conditions were acceptable according to the

current guidance document (OPP- EPA-540/9-85-005) with the exception of water hardness (150 -170 mg/L rather than the recommended 40-48 mg/L). The pH was 8.3 - 8.4, the DO ranged from 8.3 to 9.0 (84% to 100% saturation), and the temperature was 22° C. Test solutions were not aerated. Fifteen test organisms (5 in each of 3 replicates) were exposed to each phthalate. The organisms were in the second or third instar phase. The concentrations of the chemical were measured at test initiation and test termination. No mortality was observed for all three replicates at DINP test concentrations of 0.20 µl/L (nominal) and 0.12 - 0.36 mg/L measured concentrations. There was no control mortality. The study design was limited (single dose) compared to currently accepted protocol recommendations (five doses), but it does demonstrate that no mortality occurred to the midge at levels up to 0.12 mg/L DINP.

B. Marine

EG and G Bionomics (1984b) studied the acute toxicity of twelve phthalate esters to the mysid (*Americamysis bahia**) under static conditions. The basic test conditions were acceptable, with the salinity reported to be 22 parts per thousand, temperature of 22° C, and a test duration of 96 hours. The test vessels were not aerated. The data indicate that at a nominal concentration of 0.77 mg/L of DINP, with the measured treatment average of 0.39 mg/L. The chemical was not detected on Day 4, indicating the test organisms were not exposed to 0.77 mg/L continually for the 96 hours. The average concentration of DINP over the entire test was reported to be 0.39 mg/L. Only 10% mortality was observed in one of the three replicates. The other two replicates had no mortality reported. Controls had a 3% mortality rate, which is within the acceptable range. The LC50 is therefore >0.39 mg/L.

## III. Acute Freshwater Algae Toxicity

Springborn Bionomics (1984b) evaluated the phytotoxicity of 14 phthalate esters to the freshwater green alga *Selenastrum capricornutum* under static conditions. DINP concentrations were analyzed in each treatment at the beginning and at the end of each test. The results indicated that there was a slight to appreciable loss of chemical from the test solutions during the testing. Consequently, EC50 values were based on concentrations existing at the onset of the testing. The EC50 for *Selenastrum capricornutum* exposed to DINP is >2.80 mg/L.

## IV. Acute Fish Toxicity

A. Freshwater

EG and G Bionomics (1983a) studied the acute toxicity of 13 phthalate esters to the Bluegill sunfish (*Lepomis macrochirus*). For 24hr, 48hr, 72hr and 96hr DINP exposures, the NOEC was found to be <0.17 mg/L. The study was limited in that it had only one test concentration instead of the recommended 5. The nominal concentration for this study was reported to be 0.20 µl/L. The measured concentrations at test initiation were reported to be 0.13 and 0.22 mg/L at time zero, and 0.11 and 0.090 mg/L at test termination. The measured concentrations were almost double in one test tank compared tothe other test tank for the same concentration at test initiation, yet at test termination, the measured concentrations in the same two tanks were similar. This causes uncertainty about the methods used to mix and/or measure the test chemical and implies that the test results may not bereliable.

EG and G Bionomics (1983b) studied the acute toxicity of 14 phthalate esters to fathead minnow (*Pimephales promelas*) under static conditions. The LC50 for DINP was determined to be >0.14 mg/L for this species (0% mortality at this level). The data that specifically addressed DINP was not included in the submitted microfiche and thus EPA could not completely evaluate the study. There were data available in the submitted table which indicated the pH ranged from 7.2 to 7.7, temperature was 22° C, the DO ranged from 6.4 to 7.9, or 72 % to 91% saturation. This study was limited because it was only conducted at one exposure level and under static conditions. It is not advisable to conduct studies under static conditions when a chemical is insoluble in water. In this case, a follow-up study was conducted and is evaluated in the flow-through acute toxicity study discussed below.

EG and G Bionomics (1983c) studied the acute toxicity of 13 phthalate esters to fathead minnow (*Pimephales promelas*) under flow-through conditions. The LC50 is >0.19 mg/L for this test species. It should be noted that DINP was at the surface as a layer of chemical, apparently not in solution. Test conditions were in accordance with currently accepted testing protocols, with the exception that the chemical being tested did not appear to be totally in solution at the two highest test concentrations. Five nominal concentrations were used: 0.012, 0.025, 0.050, 0.10, 0.25 µg/L. Concentrations were measured at 0 hour and 96 hours, and the mean measured concentrations were observed to be: 0.019, 0.030, 0.047, 0.097, and 0.19 mg/L. Mortality for the control was 10%, exceeding the recommended 5% for flow-through studies (EPA-540-9-85-006). There was 10% mortality at a test concentration of 0.047 mg/L DINP. At the two highest test concentrations

(0.19 and 0.097 mg/L) undissolved phthalate ester was observed on the surface of the test solution at hour 0 and for the remainder of the study. No mortality was observed at 0.019 and 0.030 mg/L. The LC50 for this study is greater than 0.19 mg/L. The water solubility was reported to be 0.10-0.18 mg/L in this study which is much higher than the 0.0006 mg/L reported elsewhere in this document, indicating the measurements may not be accurate. Control samples indicated the level of detection was <0.0085 mg/L.

EG and G Bionomics (1983d) studied the acute toxicity of 14 phthalate esters to rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) under flow-through conditions. The LC50 for the rainbow trout was >0.16 mg/L. The test conditions were in accordance with currently accepted testing protocols. The pH ranged from 6.9-7.2 and water hardness ranged from 20-26 mg/L $CaCO_3$. The temperature was 11-12 °C. There were five test concentrations included in the study withtwo test replicates per concentration. Concentrations were measured at 0 and 96 hours, and were 0.16, 0.062, 0.032, 0.019, 0.0087 mg/L. No mortality was reported at any of these levels.

Adams et al. (1995) conducted the acute toxicity test of 14 phthalate esters to 8 species including sheepshead minnow (*Cyprinodon variegatus*) under both flow-through and static test conditions. The details of the test conditions were not provided. However, it is mentioned in the report that "the test procedures used in the acute toxicity tests closely followed those described in U.S. Environmental Protection Agency (EPA) methods for Acute Toxicity Tests with Fish." The solubility of DINP was 0.20 mg/L. The 96 hr LC50 was >0.52 mg/L and was obtained under static conditions. The authors concluded that "phthalate esters with alkyl chain lengths of six carbon atoms or more were not acutely toxic at concentrations up to their respective aqueous solubilities (1.1 mg/L). This lack of mortality prevented calculation of EC50 and LC50 values for the higher-molecular-weight phthalate esters for all species tested."

B. Marine

Springborn Bionomics (1984c) studied the acute toxicity of 13 phthalate esters to the sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions. DINP exerted no observable adverse effects on the minnows at concentrations up to its water solubility limit in natural seawater from a Gulf of Mexico estuary. The LC50 is >0.52 mg/L (mean measured concentration). Two concentrations were tested, one at 50% of the water solubility limit and the other at the water solubility limit. Measured concentrations of DINP were 0.21 and 0.52 mg/L. A

total of 20 fish were exposed at each treatment level and control group. The test conditions were acceptable, with the salinity equal to 19 parts per thousand, temperature25° C, pH of 8.1, and the DO of 7.3 - 7.5. Currently the OPPTS Guidelines recommend continuous exposure of the organisms to the chemical within the test vessel, however, the study authors did not follow this protocol because at the time the test methodology was not standard as it is today. Instead, the fish were removed from the test aquaria daily and were held in water removed from the respective aquarium. The test aquaria were brushed with detergent, rinsed thoroughly with freshwater, rinsed with acetone, and rinsed again with freshwater. The diluter was allowed to cycle several times before the test aquaria were allowed to fill. The fish were replaced within approximately 2 hours of removal for test vessel cleaning. The daily cleaning of the aquaria was intended to reduce phthalate-degrading bacterial populations in order to maintaindesired concentrations of phthalate esters for as long as possible. Despite these disruptions in the tanks, there was no mortality in the controls nor the treatment groups.

The Agency notes that some of the acute and chronic studies indicated that the LC50 value was above the water solubility, which limits the validity of the study, but the Agency also recognizes that the testing methodology in the 1980's is not what it is today.

## V. Chronic Toxicity Freshwater Invertebrates

Springborn Bionomics (1984d) studied the chronic toxicity of 14 phthalate esters to *Daphnia magna* under flow-through conditions. The two highest test concentrations, mean measured concentrations of 0.089 and 0.17 mg/L, contained surface film of test material throughout the study. The survival was significantly reduced at 0.089 mg/L and 0.17 mg/L, but the authors reported that this was due to entrapment of the daphnids at the surface and not necessarily to the toxicity of DINP to the daphnids. However, the daphnids at these test levels, including those not on the surface, were found to be pale and lethargic, indicating some degree of toxic effect from the chemical. The number of offspring produced were significantly reduced at concentrations greater than 0.034 mg/L. Based on the data, the NOAEL (no-observed adverse effect level) was found to be 0.034 mg/L and the LOAEL (lowest-observed adverse effect level) was observed to be 0.089 mg/L for both survival and reproductive effects, with a geometric mean of 0.055 mg/L. Therefore, the measured MATC is 0.055 mg/L.

## VI. Other Types of Data

A. Sediment Toxicity

Call et al. (2001a, b) assessed the toxicity of DINP and six other phthalate esters (PE) to freshwater benthos from exposure to treated sediment. The toxicity to freshwater invertebrates *Hyalella azteca* and *Chironomus tentans* from a 10-day exposure to treated sediment was evaluated. DINP had no effect on survival or growth of either *C. tentans* or *H. azteca*, which is consistent with the predictions based on water-only tests and equilibrium partitioning (EqP) theory, according to the study authors. No effect was observed at concentrations of 2,900 mg/kg dry weight for *Hyallela azteca* in the sediment, and 2,680 mg/(kg dw sediment) for *Chironomus tentans* in the sediment. Preliminary spiking studies were performed to assess ester stability under test conditions. The test material was reported to have a log Kow >8.0 and water solubility <0.001 mg/L. During the mixing process, samples of bulk sediment were collected on days 1, 3 and 6 after phthalate ester (PE) amendment, and pore water was separated by centrifugation. A total of five replicates of the single nominal concentration and five control replicates were included (with 10 organisms per test vessel). The PE amended test sediment was allowed to equilibrate in the flow-through test system for approximately 24 hours before animals were added. *H. azteca* (7-14 day old) and 10 *C. tentans* larvae ($2^{nd}$ and $3^{rd}$ instars, 10-12 day old) wereadded to each exposure beaker. Tests were conducted in an intermittent water renewal system. Nominal temperature was 23° C and a photoperiod of 16 hours light/8 hours dark. Each beaker contained 100 ml of sediment and 100 to 175 ml of overlying water, dependent on the stage in thesiphoning and renewal cycle. Flow-through conditions were maintained throughout the duration of the study. There were two sources for the sediment, with the mean Total Organic Carbon ranging from 2.45 to 14.1 (%). In summary, there did not appear to be any sediment toxicity from DINP to the species tested and the authors attributed this to the fact that this high molecular weight phthalate has limited water solubility (in pore water and overlying water).

B. Amphibian and Fish Toxicity

Birge et al. (1978) data was presented in the proposed technical report which was issued in September 2000. The Agency has since had the opportunity to reevaluate the data to ensure that all the necessary information could be captured, especially with the sediment toxicity studies that had been conducted by Call et al. (2001a and 2001b). These static renewal bioassays were

performed with various chemicals on some or all of the following species: rainbow trout, *Ictalurus punctatus* (channel catfish), goldfish, *Lepomis microlopus* (Redear sunfish), *Rana pipiens* (leopard frog), *Bufo fowleri* (Fowler's Toad), and the *Bufo americanus* (American toad). However, the effects of DINP were only evaluated on the channel catfish, redear sunfish, leopard frog, Fowler's toad, and the American Toad. The 4-day post-hatching LC50 (more sensitive than the 0 day) reported for channel catfish of 0.42 mg/L, Fowler's toad of 2.95 mg/L, Leopard frog of 3.63 mg/L, and the redear sunfish of 4.67 mg/L.

Chen et al. (2014) evaluated the acute toxicity and estrogenic endocrine disrupting activity of six phthalates, including DINP, using a 72hr zebrafish (*Danio rerio*) toxicity test. DINP was not tested individually. The LC50 value for the mixture of six phthalates (BBP, DBP, DEHP, DIDP, DNOP, and DINP) was found to be 0.50 mg/L. The mixture also exhibited enhanced estrogenic activity. However, the individual contribution of DINP was undetermined.

Reproductive and developmental effects of DINP (CASRN 68515-48-0) and DIDP (diisodecyl phthalate - CASRN 68515-49-1) were evaluated in Japanese medaka (*Oryzias latipes*) using a multigeneration protocol (Patyna et al. 2006) (Table 2). The $F_0$ and $F_1$ generation adults were reared to sexual maturation and the tests were concluded prior to sexual maturation of the $F_2$ generation. Phthalates were administered via a fish flake diet (Tetramin, 5% lipid content) at concentrations of 20 $\mu g\ g^{-1}$ (1 $\mu g\ g^{-1}$ fish $d^{-1}$). The endpoints evaluated were testosterone metabolism, 7-ethoxyresorufin-o-deethylase (EROD) activity, survival, development, growth, gonadal-somatic index, histopathology, sex ratio, and fecundity. Two controls were run - consisting of untreated and the acetone carrier (20 mL (1000 g Tetramin)$^{-1}$). Male fish showed a two-fold induction of several testosterone metabolites in the DINP group compared to the untreated but not the acetone control. Egg production did not differ among treatment groups. No sex- or treatment-related differences observed in the EROD assay. A significant but transient delay in red blood cell pigmentation was observed. DINP treatments had no effect across levels of biological organization (e.g., biochemical to population level).

Solyom et al. (2001) further investigated the influence of sediment-associatedphthalate esters (DEHP and DINP) on hatching and survival of the moorfrog, *Rana arvalis*. The study's primary focus was to evaluate the influence of DEHP on the hatching and survival success. DINP had been tested for one test temperature, 10° C, but there were data points that were not reported for DINP which had been reported for DEHP. This causes concern for the validity of the study. Mortality appeared to increase at concentrations of 100 mg/kg in fine sediment, though the data

did not follow a dose-response curve (there was no mortality at 300 mg/kg). An NOAEL was not achieved in this study, since there were effects at the lowest concentration tested. The data from this study was not used in this revised assessment, due to thedeficiencies.

**Table 1.** Data included in this table are what the Agency deem appropriate for a hazard assessment for the potential ecological effects of DINP to non-target ecological species.

| **Acute Toxicity** | | | | |
|---|---|---|---|---|
| **Species** | **Duration of Test** | **Experimental Type (Static/Flow - through)** | **Value mg/L - all measured concentrations unless noted** | **Reference** |
| **Invertebrates** | | | | |
| ***Freshwater*** | | | | |
| *Daphnia magna* | 48 hours | not indicated | not available | not available |
| *Daphnia magna* | 48 hours | static | LC50 0.086 | Springborn Bionomics, 1984a |
| *Paratanytarsus parthogenetica* midge | 48 hours | static | LC50 >0.12 | EG and G Bionomics, 1984a |
| **Marine** | | | | |
| *Americamysis bahia** mysid shrimp | 96 hours | static | LC50 >0.39 | EG and G. Bionomics., 1984b |
| **Algae** | | | | |
| ***Freshwater*** | | | | |
| *Selenastrum. capricornutum* Green Alga | 5 days | static | EC50 >2.8<br>EC50 >1.80 | Springborn Bionomics, 1984b<br>Adams et al. 1995 |
| **Fish** | | | | |
| ***Freshwater*** | | | | |
| *Lepomis machrochirus* bluegill sunfish | 96 hours | static | LC50 >0.17 | EG and G Bionomics, 1983a |
| *Pimephales promelas* fathead minnow | 96 hours | static | LC50 >0.14 | EG and G Bionomics, 1983b |
| *Pimephales. promelas* fathead minnow | 96 hours | Flow-through | LC50 >0.19 | EG and G Bionomics, 1983c |
| *Salmo gairdneri* rainbow trout | 96 hours | Flow-through | LC50 >0.16 | EG and G Bionomics, 1983d |
| ***Marine*** | | | | |
| *Cyprinodon variegatus* sheepshead minnow | 96 hours | flow-through<br>static | LC50 >0.52<br>LC50 >0.52 | Springborn Bionomics, 1984c<br>Adams et al. 1995 |

| | | | | |
|---|---|---|---|---|
| **Chronic Toxicity** | | | | |
| ***Freshwater Invertebrates*** | | | | |
| *Daphnia magna* | 21 days | not indicated | NOAEL= 0.0341<br>LOAEL = 0.089 for both survival and reproductive effects<br><br>MATC= 0.055 | Springborn Bionomics, 1984d |
| **Other Types of Data** | | | | |
| *Ictalurus punctatus* Channel Catfish | 0 and 4 days post hatch | Static renewal | LC50=0.87-0.42 | Birge et al. 1978 |
| *Bufo fowleri* Fowler's Toad | 0 and 4 days post hatch | Static renewal | LC50=23.51-2.95 | Birge et al. 1978 |
| *Rana pipiens* Leopard Frog | 0 and 4 days post hatch | Static renewal | LC50=4.94-3.63 | Birge et al. 1978 |
| *Lepomis microlopus* Redear Sunfish | 0 and 4 days post hatch | Static renewal | LC50=71.85-4.67 | Birge et al. 1978 |
| **Other Data** | | | | |
| Sediment Toxicity | Call et al. 2001a. No effect was observed at concentrations of 2,900 mg/kg dry weight for *Hyallela azteca* in the sediment, and2,680 mg/kg dry weight for *Chironomus tentans* in the sediment. | | | |
| *Oryzias latipes*<br>Japanese medaka | Multi-generation study ($F_0$-$F_2$) of dietary intake of DINP (1 µg/g fish/day) indicated a 2-fold induction of testosterone metabolites in males and a transient delay in red blood cell pigmentation (Patyna et al.2006). | | | |

## VII. Bioaccumulation

While there is limited experimental data on bioaccumulation factors for DINP, some field studies are available for di-2-ethyl hexyl phthalate (DEHP), a chemical similar in structure and physical-chemical properties to DINP. The majorityof bioconcentration factors (BCFs) observed for various phthalate esters fell within a range of 10 and 1000. Field studies conducted on high molecular-weight phthalates esters (i.e., DEHP, DNOP, DNNP) indicate that they do not biomagnify in aquatic food webs, demonstrating a tendency to decrease in concentration with increasing trophic levels. This is consistent with

findings from laboratory and modeling studies which indicate that metabolic transformation is a key mitigating factor.

Very limited experimental data are available on the bioaccumulation factor (BAF) of DINP. Staples et. al (1997) and Gobas et.al (2003) have reviewed the bioaccumulation of phthalate esters in aquatic food-webs. Although there were a number of bioaccumulation studies onphthalate esters, many of them suffer from experimental artifacts that make them difficult to interpret. Most of the studies are on DEHP and many are conducted with radiolabeled material without differentiating between the parent and metabolites and thus overestimate BAF.

Many studies were conducted above the solubility of the phthalate ester being tested. In order to eliminate some of these experimental artifacts, Gobas et al. (2003) plotted BCFs for aquatic macrophytes, algae, benthic invertebrates, and fish and eliminated any values where concentrations were above the known water solubility limits or were determined from studies conducted with less than a three-day exposure period. The remaining BCFs were plotted as a function of the octanol-water partition coefficient (Kow) since BCF and Kow have been shown to correlate well for many organic chemicals. The remaining data (few data points compared to the large number of experimental BCFs) do not correlate with Kow and are highly variable for individual phthalates. The high molecular weight phthalates such as DINP and DEHP have BCFs below what would be predicted by a simple correlation with Kow. Most of the data presented by Gobas et al. (2003) indicate BCFs for phthalate esters fall within a range of 10 to 1000. Using field study data, Mackintosh et al. (2004) concluded that high molecular- weight phthalates esters (e.g., DEHP, DNOP, DNNP) do not biomagnify in the aquatic food web studied and show a tendency to decrease in concentration with increasing trophic levels (Mackintosh et al. 2004). These findings are consistent with laboratory and modeling studies which indicate that metabolic transformation is a key mitigating factor for high molecular weight phthalates (Gobas et al., 2003). As indicated in Attachments A-E, the EPI Suite$^{TM}$ v4.11 model estimates BCF value ranges of 196 to 1,174 L/kg wet-wt for DINP by a regression method and 1.1 to 4.8 by Arnot-Gobas method, indicating low bioaccumulation potential. These values are based on an estimated $K_{OW}$ using a parabolic relationship and are generally appropriate for persistent chemicals (see Table 1 in Chapter 1). Since phthalates esters are easily metabolized, this relationship is probably not appropriate (Mackintosh et al. 2004).

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*Revised name is *Americamysis bahia* and has been updated from USEPA (2005) and (Environment Canada (2015)

**Attachment A: DINP, CAS Registry Number 28553-12-0**

SMILES : O=C(c1ccccc1C(=O)OCCCCCCC(C)C)OCCCCCCC(C)C
CHEM : 1,2-Benzenedicarboxylic acid, diisononyl ester
MOL FOR: C26 H42 O4
MOL WT : 418.62
---------- EPI SUMMARY (v4.11) ----+----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 9.37

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 440.16 (Adapted Stein & Brown method)
Melting Pt (deg C): 84.91 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 8.62E-007 (Modified Grain method)
VP (Pa, 25 deg C) : 0.000115 (Modified Grain method)
MP (exp database): -48 deg C
BP (exp database): 252 @ 5 mm Hg deg C
VP (exp database): 5.40E-07 mm Hg (7.20E-005 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.317e-005
log Kow used: 9.37 (estimated)
no-melting pt equation used
Water Sol (Exper. database match) = 0.2 mg/L (20 deg C)
Exper. Ref: HOWARD, PH ET AL. (1985)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.00011547 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.08E-005 atm-m3/mole (2.11E+000 Pa-m3/mole)
Group Method: 2.03E-005 atm-m3/mole (2.06E+000 Pa-m3/mole)
Exper Database: 1.49E-06 atm-m3/mole (1.51E-001 Pa-m3/mole)
For Henry LC Comparison Purposes:

User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 2.049E-002 atm-m3/mole (2.076E+003 Pa-m3/mole)
VP: 8.62E-007 mm Hg (source: MPBPVP)
WS: 2.32E-005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 9.37 (KowWin est)
Log Kaw used: -4.215 (exp database)
Log Koa (KOAWIN v1.10 estimate): 13.585

Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.8966
Biowin2 (Non-Linear Model) : 0.9946
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.5545 (weeks-months)
Biowin4 (Primary Survey Model) : 3.7017 (days-weeks )
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6960
Biowin6 (MITI Non-Linear Model): 0.7073
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.4600
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 7.2E-005 Pa (5.4E-007 mm Hg)
Log Koa (Koawin est ): 13.585
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.0417
Octanol/air (Koa) model: 9.44
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.601
Mackay model : 0.769
Octanol/air (Koa) model: 0.999

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 23.3907 E-12 cm3/molecule-sec
Half-Life = 0.457 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 5.487 Hrs

Ozone Reaction:
No Ozone Reaction Estimation

Fraction sorbed to airborne particulates (phi):
0.685 (Junge-Pankow, Mackay avg)
0.999 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 3.309E+005 L/kg (MCI method)
Log Koc: 5.520 (MCI method)
Koc : 9.479E+005 L/kg (Kow method)
Log Koc: 5.977 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 6.408E-002 L/mol-sec
Kb Half-Life at pH 8: 125.185 days
Kb Half-Life at pH 7: 3.427 years
(Total Kb applies only to esters, carbamates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 2.364 (BCF = 231.3 L/kg wet-wt)

Log Biotransformation Half-life (HL) = 0.2754 days (HL = 1.885 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.392 (BCF = 2.469)
Log BAF Arnot-Gobas method (upper trophic) = 1.142 (BAF = 13.86)
log Kow used: 9.37 (estimated)

Volatilization from Water:
Henry LC: 1.49E-006 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 806.1 hours (33.59 days)
Half-Life from Model Lake : 8965 hours (373.5 days)

Removal In Wastewater Treatment:
Total removal: 94.03 percent
Total biodegradation: 0.78 percent
Total sludge adsorption: 93.26 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
** Note: When the Log Kow is > 7, the model may be underestimating the mass of material in sediment and overestimating the mass of material in the water column (biota). Consider using the results of the default EQC model. **

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.352 | 11 | 1000 |
| Water | 16.2 | 900 | 1000 |
| Soil | 80.7 | 1.8e+003 | 1000 |
| Sediment | 2.7 | 8.1e+003 | 0 |

Persistence Time: 1.16e+003 hr

Level III Fugacity Model: (MCI Method with Water percents)

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.352 | 11 | 1000 |
| Water | 16.2 | 900 | 1000 |
| water | (0.137) | | |
| biota | (16) | | |
| suspended sediment | (0.0679) | | |
| Soil | 80.7 | 1.8e+003 | 1000 |
| Sediment | 2.7 | 8.1e+003 | 0 |

Persistence Time: 1.16e+003 hr

Level III Fugacity Model: (EQC Default)

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.128 | 11 | 1000 |
| Water | 1.87 | 900 | 1000 |
| water | (0.0012) | | |
| biota | (0.14) | | |
| suspended sediment | (1.72) | | |
| Soil | 29.3 | 1.8e+003 | 1000 |
| Sediment | 68.7 | 8.1e+003 | 0 |

Persistence Time: 3.2e+003 hr

**Attachment B: DINP CAS Registry Number 71549-78-5**

SMILES : O=C(c1ccccc1C(=O)OCCC(CCC)C(C)C)OC(CCCCC)CCC
CHEM : 1,2-Benzenedicarboxylic acid, dinonyl ester, branched
MOL FOR: C26 H42 O4
MOL WT : 418.62
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 9.30

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 433.16 (Adapted Stein & Brown method)
Melting Pt (deg C): 71.73 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 4.65E-007 (Modified Grain method)
VP (Pa, 25 deg C) : 6.19E-005 (Modified Grain method)
Subcooled liquid VP: 1.28E-006 mm Hg (25 deg C, Mod-Grain method)
: 0.000171 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.677e-005
log Kow used: 9.30 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.00019352 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.08E-005 atm-m3/mole (2.11E+000 Pa-m3/mole)
Group Method: 3.38E-005 atm-m3/mole (3.42E+000 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 9.568E-003 atm-m3/mole (9.695E+002 Pa-m3/mole)
VP: 4.65E-007 mm Hg (source: MPBPVP)
WS: 2.68E-005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 9.30 (KowWin est)
Log Kaw used: -3.070 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 12.370
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 1.0050
Biowin2 (Non-Linear Model) : 0.9991
Expert Survey Biodegradation Results:

Biowin3 (Ultimate Survey Model): 2.8528 (weeks)
Biowin4 (Primary Survey Model) : 3.9708 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6199
Biowin6 (MITI Non-Linear Model): 0.5868
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.1552
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.000171 Pa (1.28E-006 mm Hg)
Log Koa (Koawin est ): 12.370
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.0176
Octanol/air (Koa) model: 0.575
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.388
Mackay model : 0.584
Octanol/air (Koa) model: 0.979

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 25.6933 E-12 cm3/molecule-sec
Half-Life = 0.416 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 4.996 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):

0.486 (Junge-Pankow, Mackay avg)
0.979 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 3.409E+005 L/kg (MCI method)
Log Koc: 5.533 (MCI method)
Koc : 8.67E+005 L/kg (Kow method)
Log Koc: 5.938 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 4.143E-002 L/mol-sec
Kb Half-Life at pH 8: 193.642 days
Kb Half-Life at pH 7: 5.302 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 2.400 (BCF = 251.3 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.2924 days (HL = 1.961 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.452 (BCF = 2.833)
Log BAF Arnot-Gobas method (upper trophic) = 1.253 (BAF = 17.91)
log Kow used: 9.30 (estimated)

Volatilization from Water:
Henry LC: 3.38E-005 atm-m3/mole (estimated by Group SAR Method)

Half-Life from Model River: 37.53 hours (1.564 days)
Half-Life from Model Lake : 581 hours (24.21 days)

Removal In Wastewater Treatment:
Total removal: 94.03 percent
Total biodegradation: 0.78 percent
Total sludge adsorption: 93.26 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
** Note: When the Log Kow is > 7, the model may be underestimating the mass of material in sediment and overestimating the mass of material in the water column (biota). Consider using the results of the default EQC model. **

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.704 | 9.99 | 1000 |
| Water 22.2 | 360 | 1000 |
| Soil 74.9 | 720 | 1000 |
| Sediment 2.21 | 3.24e+003 | 0 |

Persistence Time: 517 hr

Level III Fugacity Model: (MCI Method with Water percents)

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.704 | 9.99 | 1000 |
| Water 22.2 | 360 | 1000 |
| water (0.219) | | |
| biota (21.9) | | |
| suspended sediment (0.112) | | |
| Soil 74.9 | 720 | 1000 |
| Sediment 2.21 | 3.24e+003 | 0 |

Persistence Time: 517 hr

Level III Fugacity Model: (EQC Default)

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.28 | 9.99 | 1000 |
| Water 3.64 | 360 | 1000 |
| water (0.00274) | | |
| biota (0.273) | | |
| suspended sediment (3.36) | | |
| Soil 29.9 | 720 | 1000 |
| Sediment 66.2 | 3.24e+003 | 0 |

Persistence Time: 1.3e+003 hr

**Attachment C: DINP CAS Registry Number 68515-48-0**

SMILES : O=C(c1ccccc1C(=O)OCCCCCCCCC)OCCCCCCCCC
CHEM : 1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich
MOL FOR: C26 H42 O4
MOL WT : 418.62

---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 9.52

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 454.14 (Adapted Stein & Brown method)
Melting Pt (deg C): 115.29 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 4.01E-006 (Modified Grain method)
VP (Pa, 25 deg C) : 0.000534 (Modified Grain method)
MP (exp database): -40 deg C
BP (exp database): 233-267 @ 4 mm Hg deg C
VP (exp database): 4.88E-06 mm Hg (6.51E-004 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 1.735e-005
log Kow used: 9.52 (estimated)
no-melting pt equation used
Water Sol (Exper. database match) = 0.00013 mg/L (20 deg C)
Exper. Ref: LETINSKI,DJ ET AL. (2002)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 4.1114e-005 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.08E-005 atm-m3/mole (2.11E+000 Pa-m3/mole)
Group Method: 1.41E-005 atm-m3/mole (1.43E+000 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.273E-001 atm-m3/mole (1.290E+004 Pa-m3/mole)
VP: 4.01E-006 mm Hg (source: MPBPVP)
WS: 1.74E-005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 9.52 (KowWin est)
Log Kaw used: -3.070 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 12.590
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 1.1135
Biowin2 (Non-Linear Model) : 0.9999
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.1512 (weeks)
Biowin4 (Primary Survey Model) : 4.2398 (days)

MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.8480
Biowin6 (MITI Non-Linear Model): 0.8750
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.4193
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.000651 Pa (4.88E-006 mm Hg)
Log Koa (Koawin est ): 12.590
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00461
Octanol/air (Koa) model: 0.955
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.143
Mackay model : 0.269
Octanol/air (Koa) model: 0.987

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 23.4075 E-12 cm3/molecule-sec
Half-Life = 0.457 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 5.483 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.206 (Junge-Pankow, Mackay avg)
0.987 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 4.677E+005 L/kg (MCI method)
Log Koc: 5.670 (MCI method)
Koc : 1.147E+006 L/kg (Kow method)
Log Koc: 6.060 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 2.854E-002 L/mol-sec
Kb Half-Life at pH 8: 281.030 days
Kb Half-Life at pH 7: 7.694 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 2.292 (BCF = 196 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.3778 days (HL = 2.387 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.042 (BCF = 1.101)
Log BAF Arnot-Gobas method (upper trophic) = 0.063 (BAF = 1.156)
log Kow used: 9.52 (estimated)

Volatilization from Water:
Henry LC: 1.41E-005 atm-m3/mole (estimated by Group SAR Method)
Half-Life from Model River: 87.05 hours (3.627 days)
Half-Life from Model Lake : 1121 hours (46.71 days)

Removal In Wastewater Treatment:
Total removal: 94.03 percent
Total biodegradation: 0.78 percent
Total sludge adsorption: 93.26 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
** Note: When the Log Kow is > 7, the model may be underestimating the mass of material in sediment and overestimating the mass of material in the water column (biota). Consider using the results of the default EQC model. **

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.933 | 11 | 1000 |
| Water 23.8 | 360 | 1000 |
| Soil 73.3 | 720 | 1000 |
| Sediment 1.97 | 3.24e+003 | 0 |

Persistence Time: 479 hr

Level III Fugacity Model: (MCI Method with Water percents)

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.933 | 11 | 1000 |
| Water 23.8 | 360 | 1000 |
| water (0.142) | | |
| biota (23.6) | | |
| suspended sediment (0.1) | | |
| Soil 73.3 | 720 | 1000 |
| Sediment 1.97 | 3.24e+003 | 0 |

Persistence Time: 479 hr

Level III Fugacity Model: (EQC Default)

| Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|
| Air 0.356 | 11 | 1000 |
| Water 3.73 | 360 | 1000 |
| water (0.00169) | | |
| biota (0.28) | | |
| suspended sediment (3.45) | | |
| Soil 28 | 720 | 1000 |
| Sediment 67.9 | 3.24e+003 | 0 |

Persistence Time: 1.25e+003 hr

**Attachment D: DINP CAS Registry Number 14103-61-8**

SMILES : CC(C)(C)CC(C)CCOC(=O)c1c(C(=O)OCCC(C)CC(C)(C)C)cccc1
CHEM :
MOL FOR: C26 H42 O4
MOL WT : 418.62
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------

Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 9.15

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 416.97 (Adapted Stein & Brown method)
Melting Pt (deg C): 86.68 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 8.2E-007 (Modified Grain method)
VP (Pa, 25 deg C) : 0.000109 (Modified Grain method)
Subcooled liquid VP: 3.21E-006 mm Hg (25 deg C, Mod-Grain method)
: 0.000427 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 3.586e-005
log Kow used: 9.15 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 0.00069953 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.08E-005 atm-m3/mole (2.11E+000 Pa-m3/mole)
Group Method: 2.94E-005 atm-m3/mole (2.98E+000 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.260E-002 atm-m3/mole (1.276E+003 Pa-m3/mole)

VP: 8.2E-007 mm Hg (source: MPBPVP)
WS: 3.59E-005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 9.15 (KowWin est)
Log Kaw used: -3.070 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 12.220
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.5288
Biowin2 (Non-Linear Model) : 0.8553
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.1303 (months)
Biowin4 (Primary Survey Model) : 3.3948 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.4623
Biowin6 (MITI Non-Linear Model): 0.0849
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.6827

Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.000428 Pa (3.21E-006 mm Hg)
Log Koa (Koawin est ): 12.220
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00701
Octanol/air (Koa) model: 0.407
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.202
Mackay model : 0.359
Octanol/air (Koa) model: 0.97

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 16.6792 E-12 cm3/molecule-sec
Half-Life = 0.641 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 7.695 Hrs
Ozone Reaction:
No Ozone Reaction Estimation

Fraction sorbed to airborne particulates (phi):
0.281 (Junge-Pankow, Mackay avg)
0.97 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 1.551E+005 L/kg (MCI method)
Log Koc: 5.191 (MCI method)
Koc : 7.162E+005 L/kg (Kow method)
Log Koc: 5.855 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 6.408E-002 L/mol-sec
Kb Half-Life at pH 8: 125.185 days
Kb Half-Life at pH 7: 3.427 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 3.070 (BCF = 1174 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.4456 days (HL = 2.79 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.678 (BCF = 4.767)
Log BAF Arnot-Gobas method (upper trophic) = 1.801 (BAF = 63.25)
log Kow used: 9.15 (estimated)

Volatilization from Water:
Henry LC: 2.94E-005 atm-m3/mole (estimated by Group SAR Method)
Half-Life from Model River: 42.83 hours (1.785 days)
Half-Life from Model Lake : 638.8 hours (26.62 days)

Removal In Wastewater Treatment:
Total removal: 94.03 percent
Total biodegradation: 0.78 percent
Total sludge adsorption: 93.26 percent

Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
** Note: When the Log Kow is > 7, the model may be underestimating the mass of material in sediment and overestimating the mass of material in the water column (biota). Consider using the results of the default EQC model. **

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.303 | 15.4 | 1000 |
| Water | 12.7 | 1.44e+003 | 1000 |
| Soil | 84.8 | 2.88e+003 | 1000 |
| Sediment | 2.19 | 1.3e+004 | 0 |

Persistence Time: 1.79e+003 hr

Level III Fugacity Model: (MCI Method with Water percents)

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.303 | 15.4 | 1000 |
| Water | 12.7 | 1.44e+003 | 1000 |
| water | (0.176) | | |
| biota | (12.5) | | |
| suspended sediment | (0.041) | | |
| Soil | 84.8 | 2.88e+003 | 1000 |
| Sediment | 2.19 | 1.3e+004 | 0 |

Persistence Time: 1.79e+003 hr

Level III Fugacity Model: (EQC Default)

| | Mass Amount (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 0.111 | 15.4 | 1000 |
| Water | 1.36 | 1.44e+003 | 1000 |
| water | (0.00145) | | |
| biota | (0.102) | | |
| suspended sediment | (1.26) | | |
| Soil | 31.2 | 2.88e+003 | 1000 |
| Sediment | 67.4 | 1.3e+004 | 0 |

Persistence Time: 4.86e+003 hr

**Attachment E: DINP CAS Registry Number 68515-53-7**
SMILES : O=C(c1ccccc1C(=O)OCCCCCCC(C)C)O
CHEM : 1,2-Benzenedicarboxylic acid, mono-C8-10-branched alkyl esters, C9-rich
MOL FOR: C17 H24 O4
MOL WT : 292.38
---------- EPI SUMMARY (v4.11) ----------
Henry LC (atm-m3/mole) : ------
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Water Solubility (mg/L): ------
Physical Property Inputs:

Vapor Pressure (mm Hg) : ------
Melting Point (deg C) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 5.22

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 404.15 (Adapted Stein & Brown method)
Melting Pt (deg C): 150.50 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 3.48E-007 (Modified Grain method)
VP (Pa, 25 deg C) : 4.64E-005 (Modified Grain method)
Subcooled liquid VP: 6.59E-006 mm Hg (25 deg C, Mod-Grain method)
: 0.000879 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 0.4715
log Kow used: 5.22 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.0524 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters-acid

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 6.74E-009 atm-m3/mole (6.83E-004 Pa-m3/mole)
Group Method: 2.81E-009 atm-m3/mole (2.85E-004 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 2.839E-007 atm-m3/mole (2.877E-002 Pa-m3/mole)
VP: 3.48E-007 mm Hg (source: MPBPVP)
WS: 0.471 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 5.22 (KowWin est)
Log Kaw used: -6.560 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 11.780
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9594
Biowin2 (Non-Linear Model) : 0.9953
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.7811 (weeks )
Biowin4 (Primary Survey Model) : 3.6626 (days-weeks )
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.8393
Biowin6 (MITI Non-Linear Model): 0.8853
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.7227
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 0.000879 Pa (6.59E-006 mm Hg)
Log Koa (Koawin est ): 11.780
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00341
Octanol/air (Koa) model: 0.148
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.11
Mackay model : 0.215
Octanol/air (Koa) model: 0.922

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 12.3161 E-12 cm3/molecule-sec
Half-Life = 0.868 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 10.421 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.162 (Junge-Pankow, Mackay avg)
0.922 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 796.6 L/kg (MCI method)
Log Koc: 2.901 (MCI method)
Koc : 949.4 L/kg (Kow method)
Log Koc: 2.977 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 3.204E-002 L/mol-sec
Kb Half-Life at pH 8: 250.370 days
Kb Half-Life at pH 7: 6.855 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 1.000 (BCF = 10 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.3604 days (HL = 0.4361 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.251 (BCF = 178.1)
Log BAF Arnot-Gobas method (upper trophic) = 2.251 (BAF = 178.4)
log Kow used: 5.22 (estimated)

Volatilization from Water:
Henry LC: 2.81E-009 atm-m3/mole (estimated by Group SAR Method)
Half-Life from Model River: 3.563E+005 hours (1.484E+004 days)
Half-Life from Model Lake : 3.887E+006 hours (1.619E+005 days)

Removal In Wastewater Treatment:
Total removal: 83.55 percent
Total biodegradation: 0.72 percent
Total sludge adsorption: 82.84 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
Mass Amount Half-Life Emissions

(percent) (hr) (kg/hr)
Air 0.054 20.8 1000
Water 16.7 360 1000
Soil 82.8 720 1000
Sediment 0.48 3.24e+003 0
Persistence Time: 764 hr

Level III Fugacity Model: (MCI Method with Water percents)
Mass Amount Half-Life Emissions
(percent) (hr) (kg/hr)
Air 0.054 20.8 1000
Water 16.7 360 1000
water (16.5)
biota (0.137)
suspended sediment (0.0197)
Soil 82.8 720 1000
Sediment 0.48 3.24e+003 0
Persistence Time: 764 hr

Level III Fugacity Model: (EQC Default)
Mass Amount Half-Life Emissions
(percent) (hr) (kg/hr)
Air 0.0435 20.8 1000
Water 11.5 360 1000
water (10.3)
biota (0.0859)
suspended sediment (1.06)
Soil 67.6 720 1000
Sediment 20.9 3.24e+003 0
Persistence Time: 949 hr

**Attachment F-DINP CAS CAS Registery Number 28553-12-0**

EPA ECOSAR Application 2.0 Organic Module Report
Results of Organic Module Evaluation

| CAS | Name | SMILES |
|---|---|---|
| 28553120 | 1,2-Benzenedicarboxylicacid, diisononyl ester | O=C(c1ccccc1C(=O)OCCCCCCC(C)C)OCCCCCC(C)C |

| Structure |
|---|

| Details | |
|---|---|
| Mol Wt | 418.62 |
| Selected LogKow | 9.37 |
| Selected Water Solubility (mg/L) | 0.2 |
| Selected Melting Point (°C) | -48 |
| Estimated LogKow | 9.37 |
| Estimated Water Solubility (mg/L) | 0 |
| Measured LogKow | |
| Measured Water Solubility (mg/L) | 0.2 |
| Measured Melting Point (°C) | -48 |

| Class Results: | |
|---|---|

Esters

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Fish | 96h | LC50 | 0.0028 | 5.0 | |
| Daphnid | 48h | LC50 | 0.0026 | 5.0 | |
| Green Algae | 96h | EC50 | 0.00035 | 6.4 | |
| Fish | | ChV | 0.000044 | 8.0 | |
| Daphnid | | ChV | 0.00021 | 8.0 | |
| Green Algae | | ChV | 0.00097 | 8.0 | |
| Fish (SW) | 96h | LC50 | 0.0027 | 5.0 | |
| Mysid | 96h | LC50 | 0.000070 | 5.0 | |
| Fish (SW) | | ChV | 0.0014 | 8.0 | |
| Mysid (SW) | | ChV | 1.5E-9 | 8.0 | |
| Earthworm | 14d | LC50 | 22.89 | 6 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

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**Attachment G-DINP CAS CAS Registery Number 68515-48-0**

Organic Module Report

Results of Organic Module Evaluation

| CAS | Name | SMILES |
|---|---|---|
| 68515480 | 1,2-Benzenedicarboxylic acid, di-C8-10- branched alkyl esters,C9-rich | O=C(c1ccccc1C(=O)OCCCCCCCCC)OCCCCCCCC |

| Structure |
|---|

| Details | |
|---|---|
| Mol Wt | 418.62 |
| Selected LogKow | 9.52 |
| Selected Water Solubility (mg/L) | 0 |
| Selected Melting Point (°C) | 25 |
| Estimated LogKow | 9.52 |
| Estimated Water Solubility (mg/L) | 0 |
| Measured LogKow | |
| Measured Water Solubility (mg/L) | |
| Measured Melting Point (°C) | 25 |

| Class results: | |
|---|---|

Esters

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|

| Class Results: | |
|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Fish | 96h | LC50 | 0 | 5 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Daphnid | 48h | LC50 | 0 | 5 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Green Algae | 96h | EC50 | 0 | 6.4 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Fish | | ChV | 0 | 8 | |
| Daphnid | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

| Class Results: | |
|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Mysid | 96h | LC50 | 0 | 5 | |
| Fish (SW) | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Mysid (SW) | | ChV | 0 | 8 | |
| Earthworm | 14d | LC50 | 20.52 | 6 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Green Algae | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Fish (SW) | 96h | LC50 | 0 | 5 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

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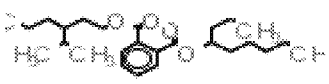
| Class Results: | |
|---|---|

**Attachment H: DINP CAS Registry Number 71549-78-5**

Organic Module Report
Results of Organic Module Evaluation

| CAS | Name | SMILES |
|---|---|---|
| 71549785 | 1,2-Benzenedicarboxylic acid, dinonyl ester, branched | O=C(c1ccccc1C(=O)OCCC(CCC)C(C)C)OC(CCCCC)CCC |

| Structure |
|---|



| Details | |
|---|---|
| Mol Wt | 418.62 |
| Selected LogKow | 9.3 |
| Selected Water Solubility (mg/L) | 0 |
| Selected Melting Point (°C) | |
| Estimated LogKow | 9.3 |
| Estimated Water Solubility (mg/L) | 0 |
| Measured LogKow | |
| Measured Water Solubility (mg/L) | |
| Measured Melting Point (°C) | |

| Class Results: | |
|---|---|

Esters

| Class Results: | |
|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Fish | 96h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Daphnid | 48h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Green Algae | 96h | EC50 | 0 | 6.4 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Fish | | ChV | 0 | 8 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Daphnid | | ChV | 0 | 8 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

| Class Results: | |
|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Green Algae | | ChV | 0 | 8 | • Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |
| Fish (SW) | 96h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |
| Mysid | 96h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |
| Fish (SW) | | ChV | 0 | 8 | • Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |
| Mysid (SW) | | ChV | 0 | 8 | |
| Earthworm | 14d | LC50 | 24.17 | 6 | • Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |

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| Class Results: | |
|---|---|

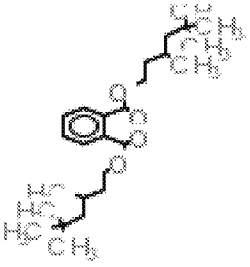
**Attachment I: DINP CAS Registry Number 14103-61-8**

Organic Module Report
Results of Organic Module Evaluation

| CAS | Name | SMILES |
|---|---|---|
| 14103618 | | CC(C)(C)CC(C)CCOC(=O)c1c(C(=O)OCCC(C)CC(C)(C)C)cccc1 |

| Class Results: | |
|---|---|

| Structure |
|---|



| Details | |
|---|---|
| Mol Wt | 418.62 |
| Selected LogKow | 9.15 |
| Selected Water Solubility (mg/L) | 0 |
| Selected Melting Point (°C) | |
| Estimated LogKow | 9.15 |
| Estimated Water Solubility (mg/L) | 0 |
| Measured LogKow | |
| Measured Water Solubility (mg/L) | |
| Measured Melting Point (°C) | |

| Class Results: | |
|---|---|

Esters

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|

Class Results:

| | | | | | |
|---|---|---|---|---|---|
| Fish | 96h | LC50 | 0 | 5 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Daphnid | 48h | LC50 | 0 | 5 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Green Algae | 96h | EC50 | 0 | 6.4 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Fish | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

| Class Results: | |
|---|---|

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Daphnid | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Green Algae | | ChV | 0 | 8 | · Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

Class Results:

| | | | | | |
|---|---|---|---|---|---|
| Fish (SW) | 96h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |
| Mysid | 96h | LC50 | 0 | 5 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported |

| Organism | Duration | End Point | Concentration (mg/L) | Max Log Kow | Flags |
|---|---|---|---|---|---|
| Fish (SW) | | ChV | 0 | 8 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

Class Results:

| | | | | | |
|---|---|---|---|---|---|
| Mysid (SW) | | ChV | 0 | 8 | |
| Earthworm | 14d | LC50 | 26.99 | 6 | • Chemical may not be soluble enough to measure thispredicted effect. If the effect level exceeds the water solubility by10X, typically no effects at saturation (NES) are reported |

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